VOLUME [illegible] NUMBER 5

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PUBLISHED MONTHLY BY AMERICAN [illegible]CAL ASSOCIATION, [illegible] NORTH DEARBORN STREET, CHICAGO, ILLINOIS. ANNUAL SUBSCRIPTION [illegible] $6.00

Entered as Second-class Matter, July 28, 1926, at the Postoffice at Chicago, Illinois, Under the Act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in the Act of [illegible], 1918, authorized Jan. 27, 1927.

CONTENTS OF PREVIOUS NUMBER

APRIL, 1933. NUMBER

ARCHIVES OF PATHOLOGY

VOLUME 15 MAY, 1933 NUMBER 5

COMPARATIVE HISTOLOGIC REACTIONS IN CUTANEOUS LESIONS INDUCED BY STREPTOCOCCI IN RABBITS PREVIOUSLY INOCULATED INTRACUTANEOUSLY OR INTRAVENOUSLY

RICHARD BÖHMIG, M.D.
Fellow of the Notgemeinschaft der deutschen Wissenschaft
AND
HOMER F. SWIFT, M.D.
NEW YORK

It has been shown that the quantitative macroscopic response of rabbits to inoculation with nonhemolytic streptococci is conditioned by the manner of previous treatment with homologous or closely related micro-organisms.[1] Animals that have had multiple focal reactions induced by repeated intracutaneous inoculations show a marked degree of hypersensitiveness, not only to the homologous strain, but to streptococci more distantly related. Previous intravenous vaccination or inoculation, on the other hand, so alters the reactivity of the tissues that subsequent intracutaneous inoculation with homologous micro-organisms often induces smaller lesions than are produced in normal animals by similar doses.[2] This is particularly true when the preliminary intravenous immunization has been prolonged and intense; but when shorter and milder intravenous treatment has been given, the intracutaneous test doses not infrequently induce slightly larger lesions than are seen in normal controls.[3]

Qualitatively, the macroscopic appearance of the two types of lesions also varies: Those of intracutaneously sensitized animals are soft and edematous in the first two or three days following inoculation, while those of intravenously immunized animals become hard and nodular within the same period following intracutaneous injection.

These macroscopic differences in cutaneous lesions of hyperergic (hypersensitive), hypo-ergic (intravenously immunized) and normal animals are striking enough to require microscopic explanation, and this communication is a report of such a histologic investigation.

But in planning the experiments a difficulty was presented which required a certain amount of manipulation. We wished to follow the

From the Hospital of the Rockefeller Institute for Medical Research.

1. Derick, C. L., and Swift, H. F.: J. Exper. Med. **49**:615, 1929.
2. Swift, H. F., and Derick, C. L.: J. Exper. Med. **49**:883, 1929.
3. Schultz, M. P., and Swift, H. F.: J. Exper. Med. **55**:591, 1932.

evolution and involution of the focal inflammation over a period of a week in areas of a size to permit practically complete excision. Doses inducing suitably sized lesions in normal animals induced too large and too destructive lesions in hypersensitive rabbits, while doses stimulating proper-sized reactions in hypersensitive rabbits gave rise to insignificant inflammation in normal animals or those that had undergone prolonged intravenous immunization. As from four to six lesions from each group were needed to give an average picture, it was unpractical to excise lesions from all groups on the same day; therefore the experiment was carried out in stages, with different sets of normal controls and with doses selected according to the previously determined reactivity of the hypersensitive or immunized groups.[4]

METHOD

Four different groups of rabbits were prepared by preliminary injections of streptococci as follows:

GROUP 1.—The animals in group 1 were sensitized by intracutaneous inoculations with living (blood broth) cultures of a strain of Str. viridans, originally isolated from a subcutaneous rheumatic nodule.[5] Individual inocula were in total quantities of 0.1 cc. During the first week the rabbits received three courses consisting of 10^{-1}, 10^{-2} and 10^{-3} cc. of culture, respectively, and subsequently one injection weekly at 10^{-3} cc. The degree of hypersensitivity was estimated from the measurements and appearance of these periodically induced lesions and also those following inoculation with 10^{-4}, 10^{-5} and 10^{-6} cc., doses which failed to produce macroscopic reactions in normal animals.

GROUP 2.—The rabbits in group 2 were immunized with the same strain of Str. viridans employed for the sensitization of group 1. The lethal dose following intravenous inoculation was approximately 5 cc. of broth culture per kilogram of body weight. The animals received weekly four intravenous injections of increasing amounts of heat-killed vaccines during the first two weeks, and then for a period of from five to ten weeks increasing doses of living cultures up to a maximum of 4 cc. The degree of antibody formation was ascertained by agglutination tests.

GROUP 3.—The rabbits in group 3 were immunized with a hemolytic streptococcus,[6] a laboratory strain originally isolated from a patient with measles, but subsequently passed through numerous mice and rabbits. The lethal dose was about 2 cc. of whole culture for a rabbit weighing 2 Kg. The mode of immunization was the same as that employed in group 2.

GROUP 4.—The rabbits in group 4 were immunized with a hemolytic streptococcus,[7] a capsulated, highly virulent strain, isolated from a guinea-pig by

4. In all instances, the adjective "hypersensitive" in the text refers to animals that had been rendered highly reactive by repeated intracutaneous inoculations with a strain of Str. viridans, and "immune" or "immunized" refers to animals that had undergone previous intravenous immunization with any strain of streptococci.

5. This is designated as Streptococcus V110A in the collection of bacteria kept in this laboratory.

6. This is designated S43 in our collection.

7. This strain, P230 in our collection, is a member of a group of streptococci recently differentiated in this laboratory by Dr. R. C. Lancefield by means of a group-specific precipitin test (J. Exper. Med., to be published).

Dr. Theobald Smith. The rabbits for eight weeks were given intravenous injections of increasing doses of heat-killed vaccine up to a maximum of the sediment obtained by centrifugating 4 cc. of culture. Intravenous inoculation with 0.25 cc. of living culture after ten weeks of vaccination resulted in so many deaths that immunization exclusively with vaccines was found necessary.

At selected intervals one or two animals from each group were tested with varying doses of streptococci given intracutaneously in order to determine the proper dose for the final testing. We aimed to induce reactions of moderate intensity, approximately from 10 to 15 mm. in diameter.[8] Then from four to eight animals of a group received from five to seven intracutaneous injections of the same testing strain, and with a skin punch measuring 11 mm. in diameter the lesions were excised daily from the first to the seventh day. Normal control animals were inoculated similarly. The lesions were so distributed that the trauma and reaction from previous operation did not vitiate the results. The excised skin, containing the underlying muscularis, was stitched to a piece of blotting paper on which orientation lines were drawn; it was then fixed in Zenker's solution containing 5 per cent acetic acid, and after suitable treatment embedded in paraffin. Guided by the orientation lines on the blotting paper, the cut was made through the center of the lesions; sections of from 5 to 10 or from 10 to 15 microns' thickness were prepared and stained with eosin and methylene blue (methylthionine chloride, U. S. P.), methyl green pyronine[9] and Gram's stain.

REACTIONS IN LESIONS INDUCED BY GREEN STREPTOCOCCI

An abstract of the records of the macroscopic and microscopic findings in the lesions in the different groups follows. The minimal, maximal and average sizes of the lesions in all are shown in table 1.

GROUP 1.—In this group, previously made hypersensitive to a strain of Str. viridans[5] and then tested with 10^{-4} cc. of the same strain intracutaneously, the histologic appearances of excised lesions were as follows:

First Day: Trace of edema; very slight swelling of capillary endothelium; very slight perivascular infiltration by polymorphonuclears and mononuclears.

Second Day: Slight edema; from moderate to marked swelling of capillary endothelium; from moderate to marked diffuse infiltration by leukocytes, in the form of phlegmons containing beginning abscesses; from few to a moderate number of monocytes, together with a greater number of other mononuclear cells.

Third Day: Marked edema; marked swelling of capillary endothelium; marked phlegmonous infiltration by leukocytes or abscess formation; elsewhere polymorphonuclears only about blood vessels; moderate number of lymphocytes and mononuclears, more of the latter than of the former.

Fourth Day: Edema, capillary endothelial swelling and infiltration by leukocytes comparable with that found on the third day, but in some lesions more

8. The hair was removed with a 0000 motor clipper in place of the depilatory previously employed.

9. Hitchcock, C. H., and Ehrich, Wilhelm: Arch. Path. **9**:625, 1930.

TABLE 1.—*Size of Cutaneous Lesions in Animals of Groups 1 to 5**

Group	Previous Treatment	Skin Test Dose, Cc.	Day	Previously Treated Group†			Group 5—Normal Controls†		
				Minimum, Mm.	Maximum, Mm.	Average, Mm.	Minimum, Mm.	Maximum, Mm.	Average, Mm.
1	Hypersensitized with Str. viridans[5]	10^{-4}	1	10 × 10 × 0.5	12 × 14 × 1	10 × 10 × 0.8	Negative	Needle prick	Negative
			2	9 × 8 × 0.5	12 × 14 × 1.5	11.1 × 11.2 × 1.7	Negative	Negative	Negative
			3	7 × 7 × 0.5	10 × 10 × 1	8.5 × 9.5 × 0.7	Negative	Negative	Negative
			4	6 × 6 × 0.5	9 × 10 × 1	8.7 × 8.8 × 0.5	Negative	Negative	Negative
			5	Pink macule	9 × 10 × 1	7.1 × 7.2 × 0.3	Negative	Negative	Negative
			6	Pink macule	7 × 7 × 0.5	..	..	..	..
			7	Pink macule	6 × 6 × 0.5	..	..	..	..
2	Immunized with Str. viridans[5]	10^{-2}	1	j.p.	10 × 12 × 0.5	6.8 × 6.4 × 0.3			
			2	j.p.	8 × 9 × 1	5.3 × 5.7 × 0.3			
			3	j.p.	8 × 9 × 1	3 × 2.9 × 0.3			
			4	Negative*	6 × 7 × 1	1.6 × 1.6 × 0.3	7 × 12 × 0.5	14 × 14 × 0.5	10.0 × 10.9 × 0.8
			5	Negative	5 × 6 × 0.5	..	5 × 5 × 0.5	11 × 10 × 1	6.3 × 6.5 × 0.5
							5 × 5 × p.	8 × 9 × p.	5.7 × 5.7 × p.
3	Immunized with hemolytic streptococcus[6]	10^{-2}	1	10 × 10 × 0.5	13 × 14 × 1	10.9 × 12 × 0.7	j.v.	8 × 9 × p.	3.2 × 3.2 × p.
			2	6 × 8 × p.	14 × 14 × 1.5	10.1 × 11.2 × 1	Negative	Negative	Negative
			3	6 × 6 × p.	12 × 14 × 0.5	8.2 × 9.3 × 0.5			
			4	7 × 8 × j.p.	11 × 13 × p.	7.1 × 7.4 × j.p.			
			5	7 × 8 × j.p.	9 × 10 × j.p.	5.7 × 6.5 × j.p.			
4	Immunized with hemolytic streptococcus[7]	10^{-2}	1	7 × 8 × 0.5	12 × 15 × 1	10.5 × 11 × 1.2	7 × 12 × 0.5	11 × 10 × 1	9.5 × 11 × 0.7
			2	6 × 7 × 0.5	13 × 15 × 1.5	10.1 × 10.7 × 1.2	9 × 10 × 0.5	11 × 12 × 1	10.8 × 11.2 × 0.5
			3	5 × 5 × p.	11 × 11 × 2	8.0 × 8.3 × 0.9	8 × 9 × p.	10 × 10 × 0.5	9.0 × 9.2 × p.
			4	5 × 5 × p.	10 × 12 × 0.5	7.5 × 8 × 0.6	7 × 7 × j.p.	9 × 10 × p.	7.7 × 8.5 × j.p.
			5	..	..	..	..	..	..
			6	Negative	7 × 10 × 1	..	Negative	9 × 9 × p.	4.0 × 4.2 × j.p.

* Tested with a green streptococcus.[5]

† The minimum and the maximum are actual measurements. The average was computed from the total number of persisting lesions. The abbreviations are as follows: p. (palpable), j.p. (just palpable) and j.v. (just visible).

disintegrated than healthy leukocytes; a slight increase of lymphocytes and mononuclears; a few perivascular histiocytes.

Fifth Day: Edema absent; capillary endothelium in places only slightly swollen, in others similar to that on the fourth day; leukocytes either absent or similar to those of the fourth day; numerous lymphocytes and mononuclears mostly perivascular in distribution; a few histiocytes.

Sixth and Seventh Days: Capillary endothelium normal; leukocytes absent or in only moderate numbers; lymphocytes and histiocytes increased, generally in the form of cuffs about the blood vessels, less marked on the seventh than on the sixth day.

GROUP 2.—These rabbits were immunized intravenously with a strain of Str. viridans [5] and tested with intracutaneous injections of 10^{-2} cc. of the homologous strain. Microscopically, the lesions on the first day were quite hard, with some increased induration during the next three days. The lesions in control animals were always soft, and pinker than those of the immune animal. The histologic appearances were as follows:

First Day: No edema; from moderate to marked swelling of capillary endothelium; from a moderate number to numerous leukocytes, many disintegrated and usually arranged in localized early abscesses; few lymphocytes; slightly more mononuclears.

Second Day: Slight edema; increased and marked swelling of capillary endothelium; numerous leukocytes about blood vessels; marked and sharply circumscribed miliary abscess formation in all lesions, containing mostly disintegrated cells; a moderate number of mononuclears, which are more numerous than lymphocytes.

Third Day: No edema; very marked swelling of capillary endothelium; abscess formation absent or moderate; numerous perivascular cuffs of histiocytes and mononuclears, also a few lymphocytes.

Fourth Day: Very marked swelling of capillary endothelium continues; two submiliary abscesses seen; marked collections of histiocytes about capillaries.

Fifth Day: In some lesions, no capillary endothelial swelling; in others, fairly well marked; thick perivascular cuffs formed chiefly of histiocytes, with a few lymphocytes.

GROUP 3.—These rabbits were immunized intravenously with a hemolytic streptococcus [6] and tested with an intracutaneous injection of a strain of Str. viridans [5] in doses of 10^{-2} cc. Macroscopically, the lesions on the first day were more indurated than those in group 2; they showed a pink color until the fifth day. The histologic appearances were as follows:

First Day: Moderate edema; from slight to moderate swelling of capillary endothelium; very numerous leukocytes, partly diffuse and partly perivascular, some disintegrated; numerous perivascular lymphocytes, in some lesions outnumbering the leukocytes; only a few mononuclears.

Second Day: Marked edema; marked capillary endothelial swelling; diffuse intense infiltration by leukocytes and mononuclears, both disseminated and perivascular.

Third Day: Edema either the same or decreasing; only moderate swelling of capillary endothelium; poorly circumscribed abscesses containing broken leukocytes; numerous lymphocytes and mononuclears in perivascular collections.

Fourth Day: No edema; very slight swelling of capillary endothelium; in two lesions were residues of abscesses, or diffusely scattered leukocytes; leukocytes absent from two; perivascular collections of lymphocytes and histiocytes somewhat diminished but still marked; only a few mononuclears.

Fifth Day: Capillary endothelium normal; no leukocytes; only moderate perivascular collections of lymphocytes and histiocytes.

GROUP 4.—These rabbits were immunized intravenously with a hemolytic streptococcus [7] in the form of vaccine and tested with intracutaneous injections of a strain of Str. viridans [5] in doses of 10^{-2} cc. Macroscopically, most of the lesions showed constantly from moderate to marked induration, but a few were soft on the first day; some had yellow centers on the first and second days. The histologic appearances were as follows:

First Day: Marked diffuse edema and capillary endothelial swelling; very numerous leukocytes, generally broken, in the form of a diffuse phlegmonous infiltration, occasionally an early abscess; a moderate number of lymphocytes and a few mononuclears.

Second Day: Marked edema; swelling of capillary endothelium more intense; large abscesses either sharply outlined or with only beginning demarcation; perivascular lymphocytes few or absent; moderate number of mononuclears and histiocytes.

Third Day: Edema less; capillary endothelial swelling diminished; sharply delimited abscesses varying in size and number; marked perivascular infiltration containing chiefly lymphocytes, fewer histiocytes and occasionally monocytes, found at a distance from the abscess, even in the muscle.

Fourth Day: Both edema and capillary endothelial swelling less; abscess formation the same; increase of lymphocytes and histiocytes in the perivascular cuffs.

Fifth Day: Edema absent; capillary endothelium normal; abscess formation only a little less; distinct decrease in cells in perivascular cuffs.

GROUP 5.—These animals were normal. They were tested with intracutaneous inocula of Str. viridans [5] 10^{-2} cc. in 0.1 cc. volume. The histologic appearances of the lesions were as follows:

First Day: Edema from slight to moderate; irregular swelling of capillary endothelium; moderate diffuse infiltration by leukocytes, partly broken; a few perivascular mononuclears.

Second Day: Edema less; capillary endothelium the same; diffuse phlegmonous infiltration by leukocytes, in places early abscess formation; a few mononuclears.

Third Day: No edema; marked swelling of capillary endothelium; leukocytes in moderate numbers, many broken and all localized about blood vessels; moderate number of mononuclears and a few lymphocytes.

Fourth Day: A few histiocytes and lymphocytes about the blood vessels, together with an occasional swollen capillary endothelial cell.

Macroscopic Comparison of Lesions.—In testing the hypersensitive animals it was necessary to employ 10^{-4} cc. of culture, that is, one one-hundredth of the amount used to induce lesions of a similar size in the three immunized groups and the normal group. The lesions in these hypersensitive animals (group 1) were usually the largest on the first day, rarely on the second; they then decreased slowly and gradually until the fifth or seventh day, when merely macules remained. In one half of the lesions lasting five days or more there occurred a slight increase suggestive of the secondary reaction described by Andrewes, Derick and

Swift.[10] In the normal controls, on the other hand, 10^{-4} cc. of culture induced no reaction or very rarely only a macule lasting one day.

In the intravenously immunized groups, the skin test dose of 10^{-2} cc. induced lesions that varied rather markedly according to the type of streptococcus used in the previous immunization. Group 2, immunized with the homologous streptococcus, showed on the first day lesions of the maximum size, which then decreased more quickly than those in the hypersensitive animals. These "immune" lesions were much harder. The same intracutaneous test dose in animals immunized intravenously with the moderately virulent hemolytic streptococcus [6] (group 3) induced somewhat larger papules, usually of maximum size on the first day, occasionally on the second. They decreased more slowly than those in group 2 and showed slightly more induration. Similar skin test doses in animals immunized with a hemolytic streptococcus [7] highly virulent for rabbits (group 4) induced the largest lesions, which decreased more rapidly, so that on the fifth and sixth days both groups that had been immunized with heterologous strains had similar lesions. Normal controls (group 5) given the same dose had larger papules than the group immunized with the homologous strain, but smaller papules than those immunized with heterologous strains, and these lesions had practically disappeared by the fourth or the fifth day.

Microscopic Comparison of Lesions.—To permit easier comparison of the reactions in the various groups of animals chart 1 is presented, showing the relative amount of each of the different components that can be easily illustrated in this manner, but with the realization that the incomplete picture thus exhibited denotes only trends.

Except for the distribution of polymorphonuclear leukocytes, the curves indicate quite clearly the intensities and time relations of the different factors entering into the composite picture of the focal inflammation, and bring out distinctly the importance of the condition of the tissues in which bacteria act. The distribution of leukocytes, however, requires additional description: These cells had a diffusely and densely scattered arrangement in the form of "diffuse infiltration" in the animals immunized with the heterologous strains on the first day, and in the normal controls and the rabbits made hypersensitive to the homologous strain, on the second day; they persisted in this arrangement in the hypersensitive group and in one group immunized with a heterologous streptococcus [6] four and three days, respectively. Leukocytes densely packed in the form of abscesses were seen at times in all of the groups—of shortest duration, one day, in the normal controls, two days in the hypersensitive group, and of longest duration in the group immunized

10. Andrewes, C. H.; Derick, C. L., and Swift, H. F.: J. Exper. Med. **44**:35, 1926.

with the highly virulent heterologous streptococcus.[7] Of the previously treated animals, the group immunized with the homologous strain showed leukocytic aggregations generally of slighter degree. A sharp demarcation of these abscesses was found only in the latter group and in the group immunized with the highly virulent heterologous streptococcus.[7] Perivascularly arranged leukocytes were seen in the hypersensitive group 1 and in the heterologously immunized [6] group 3 during

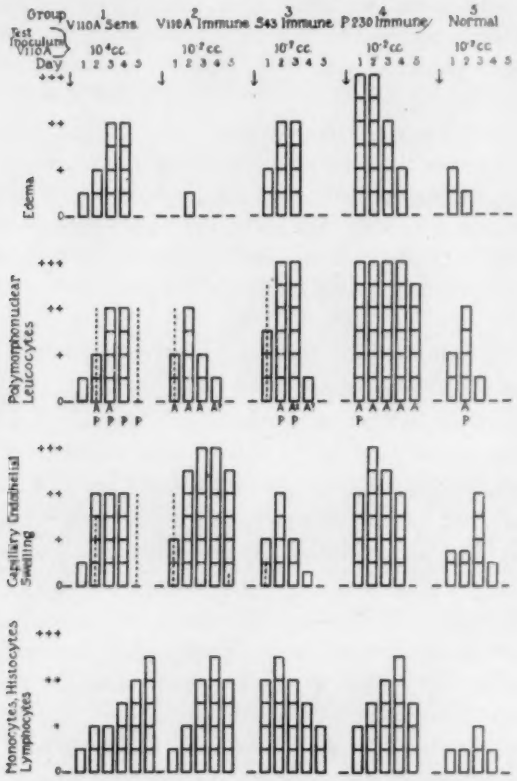

Chart 1.—Comparative amounts of the different abnormal components in the focal reactions of five groups of rabbits inoculated intracutaneously with green streptococci. Note that the hypersensitive group 1 received inocula one one-hundredth the strength of those employed in the others. The plus signs designate various intensities of reaction. Blocks represent the rule and dotted lines the exceptions. An arrow designates the day of inoculation. *A* signifies an abscess; *P*, a phlegmonous infiltration by leukocytes.

V110A designates a green-producing streptococcus (described on p. 612, under Group 1"); S 43, a moderately virulent hemolytic streptococcus (p. 612, under "Group 3"); P230, a highly virulent hemolytic streptococcus (p. 612, under "Group 4").

the beginning of the inflammation, and in the normal rabbits during its disappearance.

The comparative distribution of lymphocytes, mononuclears and histiocytes was as follows: few mononuclears in the controls and in the animals immunized with the heterologous highly virulent streptococcus [7] during the first three days; many more in the hypersensitive animals and those immunized with the homologous streptococcus during the first four and first three days, respectively. Noteworthy was the higher proportion of large mononuclears compared with that of lymphocytes in the normal and the hypersensitive groups and the group immunized with the homologous streptococcus, also the reverse condition in the groups immunized with the heterologous streptococci.

The controls showed only few perivascular histiocytes, while all the other animals had from slight to moderate collections of these perivascular cells at the end of the acute inflammation.

COMPARISON OF THE DIFFERENT GROUPS AS AN EXPRESSION OF THE INFLAMMATION AS A WHOLE

The various modes of reaction to the same stimulus but altered by the "condition of the soil" now require comment:

The normal animals showed no noteworthy reaction to intracutaneous inoculation with 10^{-4} cc. of a culture of Str. viridans. With one hundred times this dose (10^{-2} cc.), however, there occurred a moderate inflammation of four days' duration, with a maximum of edema, focal leukocytosis and capillary endothelial swelling on the first, second and third days, respectively. From the point of view both of general pathology and of comparison with other groups this succession of events is of interest, especially because in descriptions of natural or experimental inflammation one finds only summarized data and no detailed statements concerning the relationships of the individual morphologic elements in respect to time of appearance. Following injection of green streptococci of low virulence, the normal animals reacted first with a moderate local outpouring of leukocytes spreading diffusely through the corium, and these cells began to disintegrate on the first day. Thus, the chief early effect of this bacterial toxin or protein seemed confined largely to the leukocytes, for there was little edema or capillary swelling, and the latter reached its maximum only on the third day, when the leukocytes had largely disappeared. The chronological influence of the various factors in these normal animals might have been exerted in one of two ways: First, the damage of the endothelium by bacterial toxin and by the phlegmonous infiltrate might have been so intense that it could not react early. Marchand's [11] studies indicate that the emigration of leuko-

11. Marchand, F., in Krehl, L., and Marchand, F.: Handbuch der allgemeinen Pathologie, Leipzig, S. Hirzel, 1924, vol. 4, pt. 1, p. 78.

cytes depends on a damaged vessel wall. But here there was little support for this conception so far as could be seen microscopically. Because the endothelial reaction was subsequent to the leukocytic disintegration we feel that the "nonspecific" irritating influence of these dead cells had more stimulating influence on the normal vascular endothelium than did the bacterial toxin. The insignificant injury to the capillary endothelium is emphasized by the rapid disappearance of the swelling. Noteworthy also was the slight degree of histiocytic proliferation about these blood vessels.

When the same dose, 10^{-2} cc., was used in homologously hypersensitive animals, the green streptococci induced large, swollen lesions, quickly forming abscesses. Preliminary histologic studies had demonstrated the impossibility of comparing the finer tissue alterations in these lesions with those of normal or immune animals if an inoculum of 10^{-2} cc. was employed, because the reaction progressed with such rapidity that the various stages could not be followed at daily intervals. With a dose of 10^{-4} cc., which induced lesions comparable in size to those of normal or immune animals given an injection of 10^{-2} cc., the histologic reaction was more gradual in its evolution; there was a relatively marked reaction with a retardation in rate, so that edema, endothelial swelling and leukocytic emigration did not reach their maxima until the third day. This coordinated retardation among the various components in the hyperergic inflammation seems noteworthy, as is also the simultaneous diminution of these three. The coordination indicates that the irritating influence of a relatively slight streptococcic infection acted synchronously and almost equally on both the fixed and the wandering cells and on the capillary system. Comparing the heights of the curves of endothelial swelling and of leukocytes in the normal and the hypersensitive groups we see similar elevations in both groups, but broader plateaus in the latter even though it was stimulated by one one-hundredth of the dose employed in the former.

The retardation in evolution of the lesion is in contradistinction to the present conception of the reaction of sensitized tissue, and may have been the result of dosage. It may, indeed, be questioned whether it is correct to speak here of retardation, because of the appearance of the maximal endothelial reaction on the second day. If this single component is taken as the criterion of acceleration or retardation, we would speak of acceleration, because it reached its apex one day sooner than in the normal group. But the remarkable coordination in the evolution of the other factors indicates the correctness of regarding the maximal response on the third day as a true retardation, especially when compared with the normal reaction, which in animals inoculated with 10^{-2} cc. was more rapid, and which had disappeared in normal animals inoculated with 10^{-4} cc. In attempting the explanation of this interesting phenomenon on morphologic grounds alone, the question arises whether early swelling

of vascular endothelium can inhibit the exudation of edema fluid and the emigration of leukocytes. While such a relationship might possibly be deduced from the curves of the group immunized with the homologous strain, it certainly did not exist in the groups immunized with the heterologous strains, especially in group 4, in which the earliest and most marked endothelial swelling was not accompanied by a retarded outpouring of edema fluid or a slower emigration of leukocytes. On the other hand, the important rôle of the lymphatic vessels in the production of edema could not be detected by this technic.

Another possible factor in the retardation seen in group 1 may have been a true immunity to certain components of the bacteria, which manifested itself by early neutralization of some irritating toxic fraction, but because of a lack of complete neutralization of all the components the unneutralized substances remained to exert their influence at a slightly later period. Indeed, this "anti-irritative" factor is illustrated to a certain degree in the animals immunized with the homologous streptococcus. In this group, edema was even less marked than in the normal rabbits, and leukocytic emigration approximated that of the normal animals in intensity if not in duration.

Endothelial swelling and perivascular monolymphocytic reaction, on the other hand, followed in their general trend the reactions seen in hypersensitive animals, although they attained a greater intensity. In fact, a marked reaction in respect to these two factors seemed to have been a feature in all of the preliminarily treated animals, and in connection with subsequent comment it is noteworthy that the preliminarily hypersensitized group stands midway between the normal group and the group immunized with the homologous organism in the response of capillary endothelium, monocytes, histiocytes and lymphocytes. The histologic findings in both the animals immunized with the homologous streptococcus and the normal animals show, however, that neither edema and endothelial swelling nor edema and abscess formation are necessarily related, findings which have distinct interest from the point of view of general pathology.

Comparing the reactions in the groups immunized with the homologous and the heterologous strains, we find that the exudative factors were less prominent and more temporary, and the swelling of capillary endothelium more marked and prolonged, in the former. Of particular interest was the general and rapid disintegration of the leukocytes in the group immunized with the homologous organism, so that the large isolated or multiple miliary abscesses contained only broken leukocytes without necrosis of other cellular elements. This was possibly due to the higher specific bacteriotropine content of the plasma of this immune group. Under its influence the streptococci would be more quickly engulfed by the leukocytes, which would then rapidly disintegrate. Mononuclears and lymphocytes, and later histiocytes, soon invaded these

areas; this indicates the rapid development of a mechanism to insure quick healing. The gradual and continuous formation of perivascular cuffs composed of monocytes and histiocytes is also noteworthy.

In the two groups immunized with the heterologous strains interesting differences as well as similarities in reactions occurred. Among those immunized with the moderately virulent hemolytic streptococcus [6] there was seen a rapid collection of leukocytes in the form of a widespread phlegmonous infiltrate, which quickly formed poorly delimited abscesses, and these elements disappeared almost as quickly by the fourth day. But the leukocytes were more numerous than in the normal and the hypersensitive groups and the groups immunized with the homologous strain, while the degree of edema and capillary swelling in this group approximated that seen in the hypersensitive animals, even though it was of shorter duration. The parallelism in the curves of edema, capillary endothelial swelling and leukocytic emigration, while not so striking as in the hypersensitive animals, was, nevertheless, noteworthy; and, in this respect, this group stands about midway between the animals immunized with the homologous strain and the hypersensitive animals. The maximal perivascular mononuclear response was attained early, and the occurrence of perivascular monocytic and histiocytic cuffs was later. The most intense and persistent reaction was shown by the group immunized with the highly virulent heterologous streptococcus.[7] These lesions exhibited quickly the most marked edema and phlegmonous infiltration with leukocytes, and by the second day contained sharply outlined abscesses, which usually persisted in spite of the rapid disintegration of the leukocytes; but even with this persistence there was no marked lymphocytic or monocytic reaction in the neighborhood of the abscesses by the fourth day. The parallelism in the course of the edema, capillary endothelial swelling and leukocytic reaction was almost as marked as in the hypersensitive group, although it was quicker in attaining its maximal intensity in the former group.

REACTIONS IN LESIONS INDUCED BY HEMOLYTIC STREPTOCOCCI

It was planned to test rabbits previously made hypersensitive or immune as in groups 1 to 4, respectively, with intracutaneous injections of the hemolytic streptococci used in the preparation of groups 3 and 4 in order to compare their reactions to these more virulent bacteria. But for two reasons it was much more difficult to obtain comparable lesions: First, there was a variation in the total number of streptococci contained in equal quantities of different broth cultures; second, there was a marked variation in the reaction of different rabbits to the same culture; such variations have been constantly observed over a period of many years. To overcome the first objection, several control animals were inoculated with the same doses of the same culture used in the hypersensitive and immunized groups, some members of which had been

tested on the previous day to determine the proper dose. This variation in response is indicated in table 2, where the degrees of reaction shown in the different animals are indicated. Normal animals inoculated intracutaneously with small amounts of streptococcus [6] not infrequently show for from twenty-four to thirty-six hours large pink areas, which then quickly disappear. This is doubtless due to a primary toxic effect that is soon overcome, and if there is no multiplication of the streptococci, no further damage results. Larger doses, on the other hand, provoke enough local injury to require several days for its healing.

Macroscopic Comparison of Lesions.—Macroscopic differences in the reactivity of the tissues resulting from previous intracutaneous sensitization with heterologous green streptococci of low virulence, and from intravenous immunization with the same micro-organism and with a highly virulent streptococcus,[7] and finally with the homologous strain,[6] are shown in table 2. As expected, previous homologous immunization (group 8) caused the animal to react with smaller lesions than those in the normal controls. Heterologous immunization, on the other hand (groups 7 and 9), altered the soil so that there occurred larger focal reactions than in the normal controls.

In group 9, previously immunized with the very virulent hemolytic strain,[7] the reactions were so severe that necrosis was obvious to the naked eye. Likewise intracutaneous sensitization with green streptococci of low virulence made the animals of group 6 much more highly reactive to intracutaneous injection of a hemolytic streptococcus.[6]

Microscopic Comparison of Lesions.—The large size of many of the lesions made a complete survey of the inflammatory areas in the pieces of excised skin impossible; the very marked differences in intensity of reaction also made comparison more difficult than in the groups inoculated intracutaneously with green streptococci. In view of these facts, a detailed microscopic description of the different lesions is omitted, and the comparative reactions are presented simply in chart form (chart 2), where both the intensities and the time factors can be more readily compared. This chart is not comparable in all respects with chart 1, for a group previously inoculated intracutaneously with the streptococcus [6] used in skin testing was lacking here.

As anticipated, the focal reactions in normal animals following the injection of hemolytic streptococci, even of low virulence, were relatively more intense than those following similar inoculation with avirulent green streptococci. Edema was marked, capillary endothelial swelling more prolonged, and leukocytic infiltration, both in the form of diffuse phlegmons and in that of abscesses, more noticeable; the former appeared on the first day and the latter on the second, and both persisted until the fourth or the fifth day. The dissociation in maximal intensity of the various factors was less obvious here than in the normal group inoculated

TABLE 2.—*Size of Cutaneous Lesions in Animals of Group 6 to 10* *

Group	Previous Treatment	Skin Test Dose, Cc.	Day	Previously Treated Group†			Group 10—Normal Controls†		
				Minimum, Mm.	Maximum, Mm.	Average, Mm.	Minimum, Mm.	Maximum, Mm.	Average, Mm.
6	Hypersensitized with Str. viridans,[5] heterologous	10^{-4}	1	p.	14 × 17 × 0.5	8.6 × 8.4 × 0.2	Negative	19 × 20 × p.	..
			2	p.	13 × 14 × p.	6.1 × 6.6 × 0.3	Negative	Negative	..
			3	p.	10 × 12 × 0.5	4.2 × 4.2 × 0.2	Negative	Negative	..
			4	j.v.	10 × 10 × p.	3.8 × 3.8 × 0.1	Negative	Negative	..
			5	p.	10 × 10 × 1	1.5 × 1.7 × 0.1	Negative	Negative	..
			6	p.	9 × 10 × p.	..	Negative	Negative	..
7	Immunized with Str. viridans,[5] heterologous	10^{-2}	1	12 × 15 × 2	31 × 26 × 3	20 × 21.6 × 2.5	24 × 22 × 2	30 × 55 × 2.5	25.7 × 35.2 × 2.6
			2	13 × 15 × 3	31 × 42 × 2.5	19 × 21.3 × 2.7	9 × 9 × 1	11 × 12 × 0.5	10 × 10.7 × 0.7
			3	12 × 14 × 2	26 × 24 × 2	15.5 × 17 × 2.2	9 × 9 × j.p.	11 × 12 × p.	10.5 × 10 × p.
			4	9 × 9 × 1	29 × 28 × 4	18 × 17.5 × 2	Negative	p.	..
8	Immunized with hemolytic streptococcus,[6] homologous	10^{-2}	1	p.	19 × 18 × j.p.	12 × 12.4 × 0.1	6 × 6 × 0.5	17 × 15 × 1	9.6 × 9.8 × 0.6
			2	8 × 8 × p.	9 × 9 × 0.5	5 × 5 × 0.3	10 × 10 × 1	14 × 12 × 1	10.9 × 10.1 × 0.8
			3	5 × 5 × 0.5	9 × 10 × j.p.	5.3 × 5 × 0.2	6 × 7 × 0.5	14 × 10 × 1	8.2 × 7.5 × 0.7
			4	5 × 5 × p.	7 × 8 × 0.5	5.2 × 5 × 0.2	6 × 6 × 0.5	11 × 12 × 2	7.2 × 7.2 × 0.7
			5	5 × 5 × p.	7 × 6 × p.	6 × 5 × p.	6 × 6 × p.	10 × 9 × 1	5.8 × 5.3 × 0.2
9	Immunized with hemolytic streptococcus,[7] heterologous	10^{-3}	1	13 × 14 × 1	24 × 25 × 1	16 × 16.8 × 1	7 × 8 × 0.5	11 × 12 × 1	9.4 × 10.8 × 0.6
			2	11 × 12 × 1	18 × 21 × 1	14 × 15.5 × 1.5	7 × 8 × 0.5	10 × 12 × 1	9.4 × 9.6 × 0.7
			3	10 × 10 × p.	15 × 17 × 2	9.7 × 10.3 × 1.4	5 × 5 × p.	10 × 11 × 1.5	6.8 × 7.4 × 1
			4	9 × 8 × 1	13 × 13 × 1.5	8.1 × 9 × 1.4	4 × 5 × p.	9 × 12 × 1.5	4.8 × 5.8 × 0.4
			5	..	..	..	..	..	..
			6	7 × 11 × 0.5	13 × 13 × 0.5	7 × 8.2 × 0.6	Negative	9 × 9 × p.	..

* Tested with a hemolytic streptococcus.[6]

† The minimum and the maximum are actual measurements. The average was computed from the total number of persisting lesions. The abbreviations are as follows: p. (palpable), j.p. (just palpable) and j.v. (just visible).

with green streptococci; marked leukocytic emigration preceded edema and capillary endothelial swelling, which were closely associated.

Group 8, immunized with a hemolytic strain,[6] reacted to the homologous strain in some respects as group 2, immunized with a green streptococcus, did to the homologous strain (chart 1); edema and leukocytic reaction were less than in the normal animals, while capillary endothelial swelling and monohistiocytic reaction were more marked. Indeed, from distinct to marked monohistiocytic reaction seemed to be a characteristic

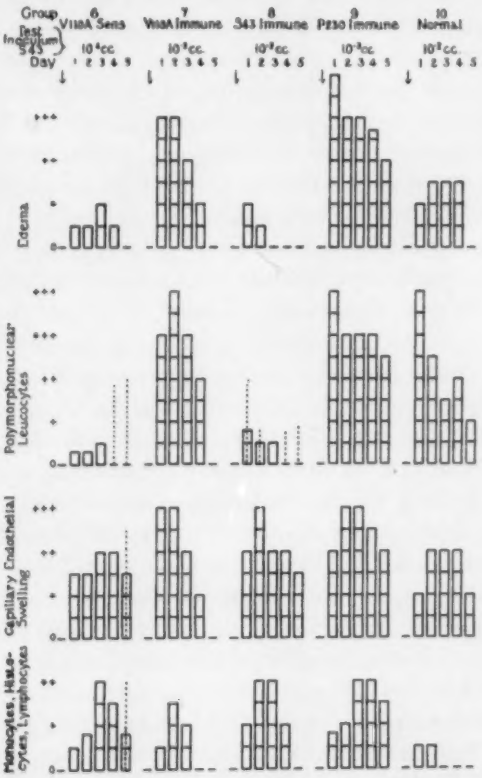

Chart 2.—Comparative amounts of the different abnormal components in the focal reactions of five groups of rabbits inoculated intracutaneously with hemolytic streptococci. Note the smaller inocula in group 6. The explanation of the chart is the same as for chart 1.

V110A designates a green-producing streptococcus (described on p. 612, under "Group 1"); S43, a moderately virulent hemolytic streptococcus (p. 612, under "Group 3"); P230, a highly virulent hemolytic streptococcus (p. 612, under "Group 4").

response of all immunized animals whether immunized with homologous or with heterologous strains, and this applies also to the hypersensitive rabbits.

The reaction of the heterologously hypersensitive group 6 to injections of 10^{-4} cc. of a hemolytic strain [6] was peculiar enough to warrant special consideration. In edema and leukocytic emigration it was less than when the comparably hypersensitive animals of group 1 (chart 1) were tested with the homologous strain of green streptococci, while in capillary endothelial swelling the reaction was similar to that of the latter group. The high degree of the capillary reaction indicates that it was a result of the state of the animal rather than of the stimulus applied. Contrary to the phenomenon observed following the testing of hypersensitive animals with homologous bacteria, that following injection of a more virulent heterologous strain showed dissociation of the various factors; but, as in the former group, there was a retardation in the curves of edema and capillary endothelial swelling, and in some instances leukocytic infiltration seemed to increase in intensity on the third and fourth days. The monohistiocytic reaction also was slow in attaining its maximal level in several animals.

Group 7, previously immunized intravenously with green streptococci, responded to intracutaneous inoculation with a hemolytic streptococcus [6] by a marked and early outpouring of edema fluid and leukocytes and by intense capillary endothelial swelling; the curves of these three then dropped in a parallel manner. Group 9, previously immunized with the very virulent hemolytic strain,[7] showed a similar rapid response of these three components, but it was even more intense in respect to edema and leukocytic reaction, both in early height of curve and in prolonged duration. The reaction of the last two groups was somewhat similar to that of the hypersensitive group 1 tested with the homologous strain, in that the various components followed a parallel course.

Summarizing, we may say, however, that, on the whole, groups 6 to 10 showed greater similarity in the course of inflammation than was apparent in the different members of groups 1 to 5. This probably is due to the fact that the strain of hemolytic streptococcus [6] used for intracutaneous injection had a much higher necrotizing and local toxic influence than had the avirulent green streptococcus.[5] This illustrates the importance of selecting suitable strains and doses of micro-organisms for bringing out the differences of reaction in immunized and hypersensitive animals, for if either the dosage is too large or the strain too destructive, all the reactions will be too accelerated and intense to permit satisfactory analysis.

COMMENT

The original object of the investigation was to determine, if possible, both quantitative and qualitative histologic differences in the focal cutaneous reactions of rabbits to intracutaneous inoculation with streptococci after the general reactivity of the animals had been altered in various ways by previous treatment.

Similar studies have been made in such focal reactions as the Arthus phenomenon (reactions to soluble proteins)[12] and Koch's phenomenon in tuberculosis.[13] Clawson[14] has recorded the histologic appearances of cutaneous lesions induced by green streptococci in normal rabbits and in rabbits previously immunized or sensitized with homologous microorganisms. The cutaneous nodules were, however, excised only on the fifth day after intracutaneous inoculation; hence no description of the evolution of the various lesions was possible. He found in hypersensitive animals a much more marked exudative reaction and abscess formation, but described the focal response in all the rabbits as a "polyblastic inflammation."

Tsuda[15] compared microscopically the lesions in normal and immune white mice resulting from subcutaneous injection of living bacteria of the "pneumo streptococcus group," and found that in the immunized animals the cellular reaction occurred more quickly and more intensely than in the normal controls, provided the immunization had not been carried too far. Macroscopic differences of focal reaction in rabbits previously inoculated intracutaneously or intravenously with nonhemolytic streptococci have been described by Swift and Derick.[16] These differences are so marked that to obtain comparable lesions suitable for excision, a hundred times the dose of the same strain of streptococci was required in the immunized and normal animals compared with the hypersensitive ones. We are aware that these differences in dosage of microorganisms introduce sources of error difficult of evaluation. But in view of the induction merely of a transitory macule in normal or immunized animals with doses (10^{-4} cc.) that induced large papules in hypersensitive animals, we could devise no way of comparing lesions of the same size other than that of varying the dosage. In addition to the quantitative macroscopic differences between the reactions of hypersensitive and normal animals, the histologic features of the two reactions varied appreciably. In the hypersensitive rabbits not only was the inflammation more intense, but there was a remarkable coordination of the reacting components, in that the high points of edema, capillary endothelial swelling and leukocytic emigration were reached almost simultaneously. There was also a retardation in the time of reaching this level compared with normal animals having lesions of the same size, in which these three components attained their maxima on different days. In comparing the entire inflammatory reaction of streptococcic

12. Opie, E. L.: J. Immunol. **9**:259, 1924. Gerlach, W.: Verhandl. d. deutsch. path. Gesellsch. **19**:126, 1923.
13. Jaffé, H., and Löwenstein, E.: Beitr. z. Klin. d. Tuberk. **50**:129, 1922.
14. Clawson, B. J.: Arch. Path. **9**:1141, 1930.
15. Tsuda, S.: Virchows Arch. f. path. Anat. **247**:123, 1923.
16. Derick and Swift.[1] Swift and Derick.[2]

hypersensitive rabbits to the homologous micro-organism and the reaction of animals sensitized to and tested with foreign protein, we find that in both the inflammation differs only in the quantity of the various factors and not in their quality. There was no "specific feature" in the lesions observed by us; and Opie,[12] Gerlach,[12] Klinge [17] and others reached the same conclusions in their investigations of the Arthus phenomenon. Maximow,[18] in an extensive discussion of the various cellular elements entering into a focal inflammatory reaction, stated ". . . the differences (in allergic reactions) as compared with the histogenesis of common inflammation are quantitative rather than qualitative." In human beings, on the other hand, the tubercle incited by Koch's bacillus, the syphilitic granuloma and the rheumatic granuloma—all three of which have been designated as "allergic reactions"—have different component cells, as brought out by supravital staining (Sabin,[19] Morgan,[20] McEwen [21]).

In the present study with streptococci in homologously immunized animals there was a dissociation in degrees of reactivity among the different factors, with a minimum of edema, a rapid disintegration of leukocytes, and a relatively rapid disappearance of miliary abscesses, which were apparently made up of collections of granulocytes with little, if any, tissue destruction. In animals previously immunized with heterologous streptococci, on the other hand, the focal response to intracutaneous inoculation was qualitatively more comparable with that of homologously hypersensitive animals, in that the curves of edema, capillary endothelial swelling and leukocytic emigration followed one another fairly closely. Of additional interest was the more intense reaction in the rabbits previously immunized with the most virulent streptococcus.[7] This was true not only in the group tested with the avirulent strain of Str. viridans,[5] but also in that tested with a more virulent hemolytic streptococcus.[6] One may, therefore, state tentatively that the reactivity of an animal is determined to a certain degree by the type of micro-organism with which it has been previously immunized. Nukada and his co-workers [22] found a somewhat comparable alteration in the response of animals to inoculation with different bacteria to be variously conditioned by the type of previous immunization. Furthermore, intravenous immunization with distantly related heterologous strains caused the animal to react to focal infection with an inflammation following the

17. Klinge, F.: Krankheitsforschung **5**:308 and 458, 1927.
18. Maximow, A. A.: Arch. Path. **4**:557, 1927.
19. Sabin, F. R.: Am. Rev. Tuberc. **25**:153, 1932.
20. Morgan, H. J.: Tr. A. Am. Physicians **45**:67, 1930.
21. McEwen, Currier: J. Exper. Med. **55**:745, 1932.
22. Nukada, S., and Kado, Y.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **67**:83, 1930. Nukada, S., and Arifuku, S.: Ibid. **70**:1, 1930.

same general qualitative course that would have occurred had it been made hypersensitive with the homologous green streptococcus.

These similarities and differences in reactivity were brought out much more clearly when an avirulent green streptococcus was used for the test injections. When, on the other hand, more virulent hemolytic streptococci were used for the test injections, the differences among the various groups were not so marked, doubtless owing to the fact that the primary injuring capacity of these more virulent strains was intense enough to mask differences in mode of reaction. Nevertheless, animals tested with the more virulent streptococci showed, among the heterologously immunized groups, inflammatory responses more intense than among normal animals. An analysis of the various inflammatory components showed them to reach a higher level and to persist longer in such heterologously immunized animals than in normal animals. One may, therefore, conclude that there is little histologic evidence of local diminished reaction in animals immunized with heterologous strains of either nonhemolytic or hemolytic varieties, but that on the contrary previous immunization with heterologous bacteria induces a reactivity of hypersensitiveness.

It should be pointed out that these statements all refer to reactions obtained with intact micro-organisms, and not with chemical fractions of bacteria or with toxic components, either endocellular or extracellular. It is possible that all the streptococci used contained some common component, and that the similarities in reaction among the different groups of animals might have been due to common responses to these common components. It is even possible that this common component may have been of the nature of a Forssman heterophilic antigen, to which all the rabbits responded with a common antibody. But in this case homologously and heterologously immunized animals would have reacted similarly, which they did not. The same criticism would apply to any other common component of the nature of a soluble protein fraction; and if the response were simply an antigen-antibody reaction as encountered in the Arthus phenomenon, the quantitative response would be expected to follow the antibody curve. The complicated antigenic mosaic of streptococci makes complete analysis of the inflammatory response impossible in the present state of knowledge, but it appears correct to assume that this response to the whole micro-organism is different from that to one or more of its components, and it may even be greater than that to the sum of these components acting independently.

SUMMARY

Rabbits previously made hypersensitive by intracutaneous injection of green streptococci were one hundred times more responsive to intracutaneous inoculation with homologous streptococci than normal or

intravenously immunized animals, when the standard measure of sensitivity was the size of the lesion.

In general, the differences shown by the various groups of animals differently inoculated were as follows:

Intravenously immunized animals responded to intracutanous inoculation with homologous streptococci with a minimum of edema and tissue necrosis, rapid focal mobilization of granulocytes, marked capillary endothelial swelling and finally late monocytic infiltration.

Animals made hypersensitive by repeated focal infections responded to small intracutaneous inoculations with either homologous or heterologous streptococci with marked edema, some tissue destruction, phlegmonous collections of granulocytes and abscesses, marked capillary endothelial swelling and marked monocytic infiltration.

Intravenously immunized animals responded to intracutaneous inoculation with heterologous streptococci with lesions larger than normal though not so large as those of intracutaneously hypersensitized controls; there was, however, distinct histologic similarity in the lesions and in the mode of appearance of the elements forming them in the last two groups of animals.

GUMMA OF THE HYPOPHYSIS AND HYPOTHALAMUS

E. B. FINK, M.D.
CHICAGO

Syphilis of the hypophysis is rare. For a long time a gumma of the hypophysis was considered a pathologic curiosity. The majority of the cases found post mortem were unsuspected clinically, while practically all the clinical cases reported have been unverified anatomically. The first case of gumma of the hypophysis was recorded by Virchow,[1] and the first histologic description was that of Weigert.[2] In 1924, Skubiszewski[3] was able to collect only twenty-five observations based on necropsies.

Involvement of the hypophysis may occur in either the congenital or the acquired form of the disease. Schmidt[4] estimated that 47 per cent of infants with congenital syphilis show pituitary changes. This is not surprising in view of the extensive dissemination of spirochetes in congenital syphilis by way of the vascular system. Cases are equally divided between the sexes. The lesions, based on differences in pathogenesis, are distinctive. In a study of twelve cases of congenital syphilis in infants, Simmonds[5] found five in which there were lesions in the hypophysis undoubtedly due to the infection. The changes consisted of more or less extensive interstitial inflammation of the anterior lobe sometimes accompanied by focal necrosis and miliary gummas. The neurohypophysis usually remains intact. Occasionally spirochetes may be demonstrated. The diffuse nature of the inflammatory reaction may be explained by the fact that in infants the lesions represent the secondary stage of the disease. Chiari[6] reported on a 9 year old girl with congenital syphilis in whom a large gumma destroyed the greater part of the anterior lobe and destroyed the posterior lobe completely. In a clinical report on congenital cerebral syphilis, Nonne[7] described

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From the Cook County Hospital and the Research Laboratory of the Chicago College of Dental Surgery, Dental Department of Loyola University.

1. Virchow, Rudolph: Virchows Arch. f. path. Anat. **15**:299, 1858.
2. Weigert, Carl: Virchows Arch. f. path. Anat. **65**:223, 1875.
3. Skubiszewski, F.: Rev. neurol. **2**:327, 1924.
4. Schmidt, M. B.: Verhandl. d. deutsch. path. Gesellsch. **6**:207, 1914.
5. Simmonds, M.: Dermat. Wchnschr. **58**:104, 1914.
6. Chiari: Deutsche med. Wchnschr. **2**:1662, 1913.
7. Nonne, M.: Deutsche Ztschr. f. Nervenh. **74**:168, 1922.

the case of a 28 year old male dwarf with infantile psyche and genitalia. Death was due to a rapidly developing tuberculous pericarditis and tuberculosis of both apexes of the lungs. There was a calcified tumor of the hypophysis. On cross-section Nonne believed it to be a gumma. There was only a small remnant of the pars anterior, and nothing was left of the pars posterior. The diagnosis of this case is questionable in the absence of a microscopic description and in the absence of other signs of syphilis. The most common calcified lesion in the region of the hypophysis is a congenital tumor. In view of the extensive tuberculosis this might have been an old calcified tubercle. Gummas do not often become calcified.

In acquired syphilis, the lesion is almost exclusively a solitary gumma varying in size from a small pea to a hazelnut. The anterior lobe is the site of predilection. As a rule the posterior lobe escapes. The process is usually not confined to the hypophysis, other portions of the brain being involved in 65 per cent of the cases. In some instances, a gummatous basal meningitis is present which spreads to the hypophysis. The infundibulum may be affected, or the process may be a downward extension from the floor of the third ventricle. A gumma may heal with the formation of a fibrous scar. In a case of diabetes insipidus which I studied,[8] the posterior lobe, infundibulum and most of the anterior lobe were replaced by fibrous tissue undoubtedly the result of a healed gumma. In a number of cases, gummas were found in the parenchymatous organs, particularly the liver. Almost 80 per cent of the cases occurred in women.

Histologically there are large areas of gummatous necrosis, at the periphery of which are found giant cells, epithelioid cells, plasma cells and rarely eosinophilic cells, outside which is a zone of granulation tissue. Frequently all that is left of the anterior lobe is a thin band of compressed cords of epithelial cells outside the gumma. Simmonds [5] found spirochetes in a gumma of the hypophysis three months after the primary infection.

Clinically, a gumma of the hypophysis is extremely difficult to recognize and can be established with certainty only by anatomic verification. The most common regional symptom is third nerve palsy. There may be temporal hemianopia due to pressure on the chiasm or primary optic atrophy. The sella may be eroded or, in rare instances, widened. Polyuria may occur if the infundibulum or tuber cinereum is involved. Glandular symptoms are usually those of hypofunction and most commonly the form of cachexia first described by Simmonds.[9] Amenorrhea is a reliable sign of hypofunction of the anterior lobe in young

8. Fink, E. B.: Endocrinology **10**:452, 1926.
9. Simmonds, M.: Deutsche med. Wchnschr. **40**:322, 1914; **44**:852, 1918.

women. According to Cohn,[10] up to 1923, a true case of dystrophia adiposogenitalis had never been proved at necropsy to be due to a gumma of the hypophysis. The case of diabetes insipidus referred to [8] was a typical example of adiposogenital dystrophy with atrophy of the thyroid gland, suprarenal glands, pancreas and sex glands.

The association of cerebral syphilis with acromegaly has resulted in considerable discussion of the possible etiologic relationship of syphilis of the hypophysis to acromegaly in a small number of cases. Most of the instances reported have been based on the amelioration of symptoms following antisyphilitic treatment, especially of third nerve palsy in acromegalic persons with signs of syphilitic meningitis. Rosenstein [11] reported the anatomic findings in a 23 year old woman with acquired syphilis and a combination of acromegaly, hypopituitarism and diencephalic symptoms. There was an eosinophilic carcinoma of the hypophysis as well as a syphilitic basal meningitis compressing the third nerve. Rosenstein [11] pointed out that it is not surprising that combinations of acromegaly and cerebral syphilis occur, because of the great frequency of cerebral syphilis. The latter may occur together with many other diseases. Moreover, syphilis of the hypophysis is a destructive process and always leads to a diminution of function, while acromegaly is an expression of hyperfunction of the gland. Esser [12] found interstitial hypophysitis with multiple miliary and submiliary gummas together with a small eosinophilic adenoma in a 66 year old woman with slight but definite acromegalic facies.

REPORT OF A CASE

The patient was a 34 year old woman who had been married four years. There were two miscarriages at the third month; there were no living children. Her complaints were pain in the occiput, which was sharp and constant, and pain in both knees. She had been unable to open her left eye for one week. Amenorrhea had been present for about a year. She also had nocturia. She appeared to be in acute pain and was semicomatose. There was severe emaciation, especially of the extremities and chest. Complete external and internal ophthalmoplegia on the left side and partial left facial paralysis were noted. On standing, the patient fell toward the left. The Romberg sign was positive. When the patient was supported in walking, the gait was ataxic. Muscular power was weak on both sides, more markedly on the left. Pain was diminished throughout the left side of the body. Examination of the fundus showed the blood vessels compressed, pale and interrupted. The disks were hazy. These changes, which were present in both eyes, were interpreted as optic neuritis. The clinical diagnosis was cerebral vascular syphilis with syphilitic meningitis of the base of the brain. The patient died in coma about a week after admission to the Cook County Hospital.

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10. Cohn, Erich: Virchows Arch. f. path. Anat. **240**:452, 1923.
11. Rosenstein, Alice: Ztschr. f. d. ges. Neurol. u. Psychiat. **88**:420, 1924.
12. Esser, A.: Centralbl. f. allg. Path. u. path. Anat. **39**:97, 1927.

The necropsy was made by Dr. C. C. Guy on the day of death. The anatomic diagnosis was: gummatous infiltration of the hypophysis, optic chiasm and base of the brain; gummatous leptomeningitis of both anterior cerebral fossae; focal encephalomalacia of the right temporal lobe; moderate edema of the brain; multiple gummas of the liver with marked syphilitic scarring (hepar lobatum); general emaciation; atrophy of the parenchymatous organs; multiple superficial decubitus ulcers of the extremities, and slight atheromatous degeneration of the aorta. Microscopically, the kidneys showed moderately advanced arteriosclerotic changes, and the suprarenal glands contained fibrous scars.

The body was that of an emaciated white woman, 145 cm. long, weighing 120 pounds (54.4 Kg.). The fat in the midline of the abdomen had a maximum thickness of about 1 cm. The suprarenal glands were small, brown and fatty. The thyroid gland and the pancreas were grossly normal.

The right occipital lobe and the posterior portion of the temporal lobe were much softer than the rest of the cerebral cortex. The brain weighed 1,000 Gm. The base of the hypophysis, when cut across, was yellow and of a nodular, cheesy consistency. In the leptomeninges there were a few glistening nodules about 2 cm. in diameter. On the under surface of the dura of both fossae there were multiple similar gummas. The sella turcica was flattened; the anterior and posterior clinoid processes were destroyed, and the cavity was filled with a gummatous growth.

After hardening in formaldehyde, the brain was of normal configuration. The convolutions were flattened, and the pia arachnoid was transparent throughout; the infundibular space was occupied by a mass stretching from the front of the chiasm to the pons, coming in contact with the uncus on the left side and obscuring the underlying structures. On the right, the oculomotor nerve wound about the mass, while on the left no trace of the third nerve could be seen. The pia was represented by a few thickened strands stretching from the pons to the dura, which was somewhat thickened but not adherent.

On longitudinal section of the brain, the mass appeared to be suspended from the recessus infundibuli. It was adherent to the adjacent optic chiasm, but did not involve the oculomotor nerve. The entire optic thalamus and the region of the cerebral peduncles were within the mass.

Microscopic examination showed the entire hypophysis replaced by a large gumma, with only a few compressed masses of anterior lobe cells near the infundibulum. There were numerous plasma cells, a few giant cells and granulation tissue at the border of the necrotic area. Spirochetes could not be demonstrated in Levaditi-stained sections of the large gummatous mass. The interpeduncular region was largely replaced by a gumma, and there was a solitary gumma in the tuber cinereum. The origin of the entire process proved to be an obliterative endarteritis of the branch of the internal carotid artery supplying the hypophysis. The smaller vessels in the base of the brain showed a marked perivascular round cell infiltration.

Clinically, this was a typical case of cerebral vascular syphilis with meningitis of the base of the brain involving the left oculomotor nerve and optic chiasm. The symptoms indicating hypofunction of the anterior lobe of the hypophysis were amenorrhea and cachexia. Necropsy disclosed, in addition, gummatous destruction of the hypophysis and interbrain. Polyuria was not present in this case in spite of the presence of a gumma in the tuber cinereum. The elements of the thalamic syndrome, central pain, hypesthesia of the left side, unstable gait and weakness of the extremities, were all recorded in the history.

Only five cases of gumma of the hypophysis have been reported in which there was an associated involvement of the diencephalon. Virchow [1] found a gumma of the hypophysis and softening in the thalamus. There were severe cachexia and bilateral ptosis, and the patient was comatose. In Westphal's [13] case there was a gumma in the left thalamus. Pozariski [14] reported a gumma of the tuber cinereum joined to a gumma of the hypophysis by a band of tissue. Goldzieher [15] found a gumma the size of a pigeon's egg occupying the position of the optic chiasm, lying over the sella and fused with the interbrain. The patient, a 32 year old man, had diabetes insipidus for six years and died with signs of increased intracranial pressure. The case of Erich Cohn [10] is similar to my case both clinically and pathologically. There was a gumma of the hypophysis, the infundibulum was thickened, and the tuber cinereum and corpora mammillare were involved in a gummatous mass. Clinically there was a combination of adiposogenital dystrophy and cachexia. Cohn stated his belief that in these cases there is a downward extension of the process from the floor of the third ventricle to the hypophysis, but in view of the close relationship between the blood supply of the hypophysis and that of the floor of the third ventricle, it seems more probable that the entire hypophyseodiencephalic area is affected from a common vascular source.

SUMMARY AND CONCLUSIONS

Gumma of the hypophysis is rare, less than thirty cases having been reported. In only five was there gummatous involvement of both the hypophysis and the interbrain.

In the presence of signs of cerebral syphilis, the occurrence of hypopituitary symptoms such as severe cachexia, amenorrhea or pituitary dystrophy justifies the diagnosis of gumma of the hypophysis. Polyuria indicates involvement of the infundibulum or of the tuber cinereum.

Tremor, weakness of the extremities, unstable gait, atrophy of the extremities and hypertonia may occur when the thalamus is destroyed by a gumma.

Acromegaly cannot be caused by syphilitic involvement of the hypophysis, because syphilis always produces destructive effects in parenchymatous organs, whereas acromegaly is an expression of hyperfunction of the hypophysis.

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13. Westphal, C.: Allg. Ztschr. f. Psychiat. u. psychiat.-gerichtl. Med. **20**:481, 1863.

14. Pozariski, cited by Skubiszewski.[3]

15. Goldzieher, M.: Verhandl. d. deutsch. path. Gesellsch. **16**:281, 1913.

WEIGHTS OF VARIOUS ORGANS IN THE ADULT RAT AFTER INANITION WITH OR WITHOUT THE DIETARY ACCESSORIES

CHARLES E. McLENNAN, M.A.
AND
C. M. JACKSON, M.D.
MINNEAPOLIS

The effects of inanition on organ weights are important in human pathology as well as in the interpretation of animal experiments on malnutrition. However, satisfactory quantitative data on this subject are comparatively rare, even for the animals most widely used in nutritional research. For the adult rat, the study of Jackson [1] is the most extensive, but is inadequate in several respects. The present investigation was therefore undertaken, with statistical methods, adequately controlled, on rats of known ancestry and uniform environment. The purpose was to establish the changes in weight of the various parts, systems and organs when the loss in body weight has reached 40 per cent on water alone. Further experiments were devised to ascertain whether the observed changes in weight could be ascribed to the absence of the dietary accessories (protein, salts, vitamins). Prof. George O. Burr gave us assistance in planning the diets used.

MATERIAL AND METHODS

In the present study, sixty nearly adult male rats were used. They were of the Wistar albino strain, reared in the colony at the Institute of Anatomy. The animals were well nourished on a (McCollum) stock diet consisting of whole ground wheat, 67.5 per cent (by weight), commercial casein 15, whole milk powder 10, butter 5.2, calcium carbonate 1.5, and sodium chloride 0.8. One per cent of cod liver oil was added to the mixture before feeding.

The sixty rats were taken for experiment when they had reached a body weight of about 240 Gm., the age being from 3 to 4 months. They were divided into four groups of fifteen each, litter mates being distributed among the various groups so far as possible.

One group served as normal controls. These rats were killed and examined immediately. Another group of the rats was given merely tap-water (city supply) ad libitum until each rat had lost about 40 per cent in weight. The third group was kept on a daily ration devised to cover merely the normal maintenance requirements of protein, vitamins and salts, as follows: commercial casein, 2 Gm.; powdered yeast and wheat germ (mixture of equal parts by weight), 0.7 Gm.; cod liver oil, 3 drops; salt mixture (McCollum 185), 0.5 Gm.; tap-water ad libitum. Since this

From the Department of Anatomy, University of Minnesota.

1. Jackson, C. M.: Am. J. Anat. **18**:75, 1915.

ration is insufficient in calories, the animals lost weight, but at a slower rate than did the rats given only tap-water. The rats in the fourth group were each fed daily 2.71 Gm. of a mixture of cornstarch and cane sugar, equal parts by weight, with tap-water ad libitum in addition. This mixture contained practically none of the dietary accessory factors, but was equivalent in energy (caloric value) to the diet used for the third group.

Throughout the experiment, the rats were placed in a room of constant temperature, within the range from 78 to 80 F. (25.6 to 26.7 C.), in individual cages with wire-net bottoms. Individual records were kept of daily weight and water consumption, including the water used in mixing the diets to a paste.

At the close of the experiment, the rats were killed by chloroform and were carefully dissected, in general according to the technic described by Jackson [2] for the various organs and parts. The entire skeleton and musculature were first weighed together. Then similarly the combined skeleton and musculature of the left thigh were weighed and likewise the corresponding segment in the forelimb. Finally, the left femur and humerus were carefully cleaned by dissection and weighed separately (fresh, without drying). The weight of the corresponding limb muscles was obtained by difference, thus giving samples for both skeleton and musculature.

The mean measurements with the probable errors for the various groups were calculated as shown in the accompanying table, using the statistical formulas of Dunn.[3] In comparing any two means, the "significance ratio" (difference divided by the probable error of the difference) is used. In general, if this ratio is above 3, the difference is assumed to be significant, with less than one chance in twenty that it is merely the accidental result of random sampling. If the ratio is between 2 and 3, the difference is of more doubtful significance; and if it is below 2, the difference is considered as insignificant. Further details, with a tabulation of the individual observations, are included in a thesis by McLennan.[4]

GENERAL OBSERVATIONS

Age.—The average final age of the controls (table) was 106.6 days; that of rats given the tap-water only and those given the energy diet varied from 105.9 to 118.6 days. These differences in age are statistically insignificant.

Duration of Tests.—The mean duration of the inanition period required for the loss of 40 per cent in body weight in the rats on tap-water was 12.27 days (range from 10 to 16 days). The mean duration was significantly extended to 17.33 days (range from 12 to 22) in the group on the energy diet, and to 20.27 days (range from 12 to 29) in the group on the dietary accessories. The difference between the latter two groups is statistically insignificant. The addition of the dietary accessories therefore apparently had no appreciable specific effect (aside from their caloric value) in prolonging the duration of inanition.

2. Jackson, C. M.: J. Nutrition **3**:61, 1930.

3. Dunn, H. L.: Physiol. Rev. **9**:275, 1929.

4. McLennan, C. E.: The Effects of Underfeeding upon the Weights of the Various Parts, Systems and Organs of Adult Albino Rats, unpublished thesis for the degree of Master of Arts, University of Minnesota, 1932.

The average inanition period of 12 days in the rats given only tap-water was distinctly longer than that of 9 days observed by Jackson [1] in acute inanition with loss of about 34 per cent in body weight. It also exceeded the average survival period of 7 days noted by Osborne and Mendel [5] in six rats with an average initial body weight of 215 Gm. Barlow and Whitehead [6] found that the weight changes in rats on water alone were roughly proportional to the length of the inanition period of from 2 to 12 days. Chanutin and Shearer [7] in extreme cases obtained losses of from 40 to 50 per cent in fasting white rats. Horst, Mendel and Benedict [8] concluded that the environmental temperature is an important factor in determining the survival period of fasting animals. Their rats fasting at 26 C. (near the critical temperature at which metabolism is lowest) lived an average of 16.5 days, with loss of 49 per cent in body weight; while those at 16 C. survived only 11 days, with a loss of 44 per cent. Lilly and Newburgh [9] found that rats on distilled water died in 9 or 10 days, losing from 37 to 41 per cent in weight; while rats on a diet of vitamin-free casein, starch, fat and salts lived 41 days, with an average loss of only 22.5 per cent in body weight.

Water Consumption.—The daily water consumption of 6.20 cc. by the rats given tap-water only does not differ significantly from that of 5.70 cc. by the group on the diet of starch and sugar (table). However, the consumption of 10.28 cc. by the group on the dietary accessories is significantly higher. This is probably due to the saltier character of that diet. Yet the rats fed the dietary accessories showed a water consumption only about half as great as that observed by Jackson and Smith [10] in adult rats on a normal diet. This is in accordance with the principle that the water consumption tends to be proportional to the food intake.

General Appearance.—The normal control rats were well nourished and healthy in all respects.

The rats given only tap-water in general showed externally more severe effects than the other two test groups, although all suffered the same loss of 40 per cent in body weight. Of the fifteen rats on tap-water, eleven at necropsy presented no external abnormality, aside from moderate emaciation. Three others appeared extremely emaciated, with roughened coats of hair, and one was moribund, with hematuria. Six additional rats on tap-water died unexpectedly with losses of from 34 to

5. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem. **29**:289, 1917.
6. Barlow, O. W., and Whitehead, R. W.: Am. J. Physiol. **89**:548, 1929.
7. Chanutin, A., and Shearer, L. D.: J. Biol. Chem. **91**:475, 1931.
8. Horst, K.; Mendel, L. B., and Benedict, F. G.: J. Nutrition **3**:177, 1930.
9. Lilly, C. A., and Newburgh, L. H.: Proc. Soc. Exper. Biol. & Med. **28**:690, 1931.
10. Jackson, C. M., and Smith, V. D. E.: Am. J. Physiol. **97**:146, 1931.

39 per cent in body weight, and are excluded from the tabulated data. In the other test groups, none died before the end of the experiment.

Of the fifteen rats on the dietary accessories, thirteen showed no especial abnormality, aside from emaciation. One developed marked kyphosis, with slight nasal hemorrhage, and one became too weak to consume the last daily ration.

The rats on the energy diet (starch and sugar) appeared in general to be in better physical condition than either of the other test groups. Fourteen remained active and normal in appearance, except for moderate emaciation. One finally became weak and inactive. No signs of specific deficiency disorder were observed in any of the three test groups.

BODY AND TAIL LENGTHS

As shown in the table, the body (nose-anus) length presents small, though statistically significant, decreases of from 3 to 5 per cent in the three test groups, in comparison with the normal controls. The differences between the test groups, however, are of questionable significance.

The changes in tail length are still slighter, only that of —3 per cent in the group given tap-water probably being significant. Since the tail length shrinks less than the body length, the test rats become relatively long-tailed individuals. These results on body and tail lengths are in agreement with those of the earlier experiments of Jackson.[1]

HEAD, VISCERA AND REMAINDER

Head.—The head, with losses of from 18 to 20 per cent, shows no significant differences among the test groups. The decrease in the head is, of course, the resultant of that for its various component organs. In some of these (brain, eyeballs, skeleton), the loss is relatively slight; in others (muscles, integument, salivary glands), considerably greater. Jackson [1] likewise concluded that during inanition in the adult rat the head loses relatively less than the whole body.

Visceral Group.—The visceral group includes the various weighed organs, exclusive of integument, skeleton and musculature. The total weight of the viscera (calculated from the data in the table) is 29.36 Gm. for the normal controls, 17.45 Gm. for the rats given tap-water, 17.86 Gm. for the rats on the dietary accessories, and 17.54 Gm. for the rats on the energy diet. The total visceral group therefore remains nearly unchanged in relative weight, forming about 12 per cent of the body weight in both controls and test groups. This is in fair agreement with the results of Jackson.[1] Although the visceral group, as a whole, thus decreases in proportion to the body weight, with loss of about 40 per cent in the various test groups, there is great variation among the individual organs, as will be shown later.

Remainder.—The "remainder" is the amount obtained by subtracting from the body weight the weight of the skin, skeleton, musculature, visceral group and gastro-intestinal contents. It includes the adipose tissue and a few small, unweighed organs (notably the tongue, trachea, larynx and bladder), as well as the loss by evaporation and escape of liquids. The average weight of the remainder in the normal controls (as calculated from data in the table) is 38.43 Gm., or about 16 per cent of the body weight. In the rats given tap-water it is 11.96 Gm.; in the rats on the dietary accessories, 11.84 Gm., and in those on the energy diet, 12.24 Gm. These figures indicate that the remainder in each of the test groups has lost about 69 per cent in weight, or relatively much more than the body as a whole. These results are in general agreement with those of Jackson,[1] who ascribed the great loss in the remainder chiefly to decrease in fat.

In the following pages the various organs will be considered in the approximate order of their relative losses in weight during the inanition tests. On this basis they may be divided into four groups, as shown in the table.

ORGANS REMAINING NEARLY CONSTANT IN WEIGHT

This group of organs (table) includes several showing little or no loss in weight, although the corresponding body weight had decreased 40 per cent in each of the tests. In general, the changes in this group remain within a range of 10 per cent above or below the normal weights.

Stomach.—Although the gastro-intestinal tract as a whole lost heavily in weight, the stomach appeared remarkably resistant. In the rats on tap-water there was no loss but an average increase of 14 per cent, and in those on the energy diet an increase of 9 per cent, while in the rats on the dietary accessories there was a slight loss of 10 per cent in the weight of the stomach. This result is difficult to explain. The intestines, as will be shown later, suffer a much larger loss, with no appreciable difference between rats on tap-water and those on the dietary accessories.

Suprarenal Glands.—The apparent increase of 12 per cent in the suprarenal glands of the rats on tap-water and that of 9 per cent in the rats on the dietary accessories are of somewhat doubtful statistical significance (with significance ratios of 2.4 and 2.2), while the rats on the energy diet show practically no change (+1 per cent). These results are in fair agreement with those of Jackson,[1] who found an apparent gain of 2 per cent in the suprarenal glands of adult rats during acute inanition, and a loss of only 9 per cent during chronic inanition. Various investigators have shown that under certain conditions of inanition these glands may increase notably in weight.

Spinal Cord and Brain.—The average weight of the spinal cord showed slight gains in all three test groups, but the increase is statistically significant only in the group on the energy diet (+8 per cent). Jackson [1] likewise found practically no change in the weight of the spinal cord in rats during acute inanition, and a loss of only 4 per cent during chronic inanition.

The brain in the present series exhibits a slight tendency to loss, varying from 2 per cent in the rats on the dietary accessories to 5 per cent in those on the energy diet. Only the latter is statistically significant. Here also the results agree fairly with those of Jackson,[1] who found a loss of 5 per cent during acute inanition, and 7 per cent during chronic inanition. It is evident that the central nervous system suffers little or no loss in weight during inanition.

Eyeballs.—The eyeballs agree with the nervous system in their remarkable resistance to loss in weight during starvation. In all three test groups of the present series, their change in average weight did not exceed 1 per cent. This is in accordance with general experience, although in the earlier experiments of Jackson [1] there was an apparent loss in eyeball weight of about 4 per cent in acute and 6 per cent in chronic inanition.

Humerus and Femur.—Only the left humerus and femur were taken as samples of the skeleton in the present study. The humerus lost 5 per cent in the group on tap-water, and showed insignificant losses of 1 and 2 per cent in the other groups. The femur showed small but significant losses, varying from 4 per cent in the rats on the energy diet to 7 per cent in the rats on tap-water. While these samples might not truly represent the entire skeleton, the results are in fair agreement with those of Jackson,[1] who found no appreciable change in the weight of the entire ligamentous skeleton (including ligaments and cartilages) during acute inanition, and an apparent gain of about 2 per cent in chronic starvation. The resistance of the skeleton to loss during simple inanition is in accordance with general experience.

The combined weight of the skeleton and musculature in the present series will be considered later.

ORGANS WITH LOSS RELATIVELY LESS THAN THAT OF THE BODY

This group includes several organs with losses ranging in the tap-water tests from 15 to 32 per cent, or definitely less than the 40 per cent loss in body weight (table). The combined weight of skeleton and musculature falls into this group, but will be considered with the muscles in the next group.

Testes and Epididymides.—The testes suffered a significant loss in all three test groups, amounting to 15 per cent in the rats on tap-water and in those on the energy diet and to 21 per cent in those on the dietary

accessories. When the latter group is compared with the other two, the difference approaches that required for statistical significance (ratio 2.9). But the difference is difficult to explain. The greater loss in the group on the dietary accessories can scarcely be attributed to the longer period of inanition, since the time was nearly as long in the group on the energy diet. In the earlier experiments, Jackson [1] found a decrease of from 30 to 40 per cent in the weight of the testes, and concluded that this loss is relatively similar to that of the entire body.

In the epididymides the decrease in weight was relatively greater than in the testes. The loss in the epididymis in the group on the tap-water was 30 per cent. Apparently the loss in the group on dietary accessories (32 per cent) was slightly greater, and that in the group on the energy diet (25 per cent) somewhat less; but these differences do not prove to be statistically significant.

Lungs.—The decrease of 26 per cent in the weight of the lungs in the rats on tap-water is not significantly greater than that of 22 per cent in the other two groups. However, one perhaps might expect to obtain heavier lungs in the latter groups, owing to greater chance of infection in the longer test periods. Jackson [1] found somewhat greater losses of from 31 to 40 per cent in the weight of the lungs during inanition in adult rats.

Integument.—The integument (including skin, external ears, hair and claws) showed losses of from 29 to 31 per cent in the three test groups, with no significant differences among them. Jackson [1] found similarly a loss of from 31 to 38 per cent in the weight of the integument, although in these earlier experiments the decrease in body weight was only from 33 to 36 per cent.

Hypophysis.—The hypophysis lost 30 per cent in weight in the group on tap-water. The slightly greater decreases of 35 per cent in the group on the dietary accessories and of 31 per cent in the group on the energy diet are not significantly different. In the earlier inanition experiments, with loss of from 33 to 36 per cent in body weight, Jackson [1] noted a decrease of 26 per cent in the hypophysis.

Kidneys.—The kidneys suffered a loss of 32 per cent in the group on the tap-water, and of 33 per cent in that on the energy diet. In the rats on the dietary accessories the loss (26 per cent) is significantly lower, indicating that in some way the presence of these dietary factors slightly protects the kidneys from loss in weight under these conditions. Jackson [1] found a loss of about 26 per cent in the kidneys when the body loss was 33 per cent in acute, and 36 per cent in chronic inanition.

ORGANS WITH LOSS NEARLY PROPORTIONAL TO THAT OF THE BODY

This group includes several organs with losses ranging from about 35 to 45 per cent, which is within 5 per cent of the loss in body weight (table).

Thyroid Gland.—The loss of 33 per cent in the weight of the thyroid gland in the group on tap-water is apparently exceeded slightly by a loss of 38 per cent in the group on the dietary accessories and also in that on the energy diet. It might be concluded that this apparently greater loss is due to the longer duration of the tests with the dietary accessories and the energy diet. However, the thyroid gland is so variable in weight that the difference of 5 per cent is statistically insignificant. In the earlier experiments, Jackson [1] found no apparent change in the thyroid gland during acute inanition, but a loss of 22 per cent in chronic inanition. The unusual difficulty in the technic of dissection makes the weight of this organ in the rat somewhat unreliable.

Heart.—The loss of heart weight in the rats on tap-water was 35 per cent. In the group on the dietary accessories the loss was 39 per cent, the difference being barely significant (ratio 3.2). However, the loss of 37 per cent in the group on the energy diet does not differ significantly from that of the other test groups, which makes the interpretation of the results doubtful. In general, there appears to be a tendency to greater loss in cardiac weight during longer periods of inanition. This is in accordance with the conclusions of Jackson,[1] who found a loss of 28 per cent in acute, and of 33 per cent in chronic inanition (losses in body weight, 34 and 36 per cent, respectively).

Skeleton and Musculature.—The weight of the total skeleton and musculature (combined) lost from 31 to 32 per cent in the test groups, or relatively less than the whole body. However, as was shown for the humerus and femur, the skeleton decreased but slightly, so it follows that the loss for the musculature must have been greater than is indicated by the combined weight.

The loss shown for the sample muscles of the limbs (arm and thigh) is 35 per cent in the group on tap-water. The decreases of 39 and 40 per cent in the groups on the energy diet and the accessories diet, respectively, are significantly greater. This apparently justifies the conclusion that the musculature suffers a greater loss during inanition of longer duration, but that the result is not materially affected by the presence or absence of the accessory dietary factors. Jackson [1] similarly found a loss of 31 per cent in the musculature during acute, and of 41 per cent during chronic, inanition, with a loss of 34 or 36 per cent in body weight.

Submaxillary Glands.—In the group on the dietary accessories, the loss of 35 per cent in the weight of the submaxillary glands appears definitely less than that of 43 per cent in the group on tap-water; while the loss of 51 per cent in the group on the energy diet is significantly greater. In this case, we may apparently conclude that a longer period of inanition (with the same loss in body weight) tends to increase the loss in the submaxillary gland, but that this tendency is more than counterbalanced by the protective effect of the accessory factors in the diet.

Prostate.—The prostate lost 45 per cent in weight in the rats on tap-water and 47 per cent in the rats on the dietary accessories. In comparison with these two groups, it is doubtful whether the loss of 36 per cent in the rats on the energy diet is significantly lower (the significance ratios are 2.2 and 2.7, respectively). No data for comparison are available in the literature.

ORGANS WITH LOSS RELATIVELY GREATER THAN THAT OF THE WHOLE BODY

With the exception of the spleen and thymus, the organs in this group belong to the digestive tract.

Intestines and Contents.—The weight of the empty intestines lost 47 per cent in the group on tap-water. The decreases of 44 per cent in the group on the dietary accessories and of 50 per cent in the group on the energy diet are but insignificantly different. However, when the latter two groups are compared with each other, the difference is questionably significant (ratio of 2.5). The results therefore indicate that the accessory factors possibly tend to protect the intestines from loss in weight during inanition. In the earlier studies of Jackson,[1] the intestines were not weighed separately, but the stomach and intestines combined showed an apparent loss of 57 per cent in both acute and chronic inanition. In the case of the intestines, the results are subject to considerable error on account of difficulty in the technic of removal of the contents.

As might be expected, the gastro-intestinal contents decreased markedly during inanition (the gastric and intestinal contents not being measured separately). The loss of 65 per cent in the rats on the energy diet is significantly less than that of 70 per cent in the rats on tap-water, while that of 54 per cent in the rats on the dietary accessories is still lower. The retention of a larger mass of intestinal contents in the latter group may be associated with the greater water intake of this group, which in turn (as mentioned) may perhaps be due to the more salty character of the diet.

Spleen.—The loss in splenic weight is 50 per cent in the rats on tap-water. The decrease is but insignificantly lower in the rats on the dietary accessories (46 per cent) and higher in the rats on the energy diet (52 per cent). The situation is therefore somewhat similar to that for the intestines. In the case of the spleen, however, the probability of significance in the difference between the group on the dietary accessories and that on the energy diet is lower. The significance ratio of 1.9 indicates about one chance in four that this difference is merely the accidental result of random sampling. For an organ so variable as the spleen, the number of cases must be large, or the differences great, in order to obtain a significant result.

Mean Data, with Probable Errors, for the Four Groups of Fifteen Rats Each

The data are expressed in grams (unless otherwise specified). The values were obtained at necropsy, except that of water consumption. The figures in parentheses indicate the percentage changes in the various test groups, in comparison with the normal controls of the first column

	Normal Controls	Rats on Tap Water	Rats on Dietary Accessories	Rats on Starch and Sugar (Energy Diet)
Age, days	106.6 ± 5.2	105.9 ± 3.6	109.5 ± 3.5	118.6 ± 4.0
Inanition, days	.....	12.27 ± 0.32	20.27 ± 0.86	17.33 ± 0.58
Water daily, cc	.....	6.20 ± 0.31	10.28 ± 0.37	5.70 ± 0.18
Body weight	240.60 ± 0.27	144.53 ± 0.45 (—40	144.20 ± 0.37 (—40)	143.40 ± 0.37 (—40)
Body length, cm	21.347 ± 0.057	20.220 ± 0.085 (—5)	20.487 ± 0.062 (—4)	20.720 ± 0.112 (—3)
Tail length, cm	19.71 ± 0.13	19.19 ± 0.11 (—3)	19.79 ± 0.17 (+1)	19.61 ± 0.13 (—1)
Head	20.16 ± 0.022	16.11 ± 0.15 (—20)	16.45 ± 0.15 (—18)	16.37 ± 0.094 (—19)
Organs remaining nearly constant in weight				
Stomach	0.919 ± 0.020	1.049 ± 0.023 (+14)	0.829 ± 0.015 (—10)	0.998 ± 0.034 (+9)
Suprarenal glands	0.0284 ± 0.0007	0.0318 ± 0.0012 (+12)	0.0308 ± 0.0008 (+9)	0.0287 ± 0.0008 (+1)
Spinal cord	0.5379 ± 0.0087	0.5604 ± 0.0115 (+4)	0.5501 ± 0.0068 (+2)	0.5801 ± 0.0087 (+8)
Eyeballs	0.2732 ± 0.0040	0.2692 ± 0.0036 (—1)	0.2723 ± 0.0029 (—0)	0.2701 ± 0.0056 (—1)
Brain	1.889 ± 0.017	1.831 ± 0.018 (—3)	1.847 ± 0.014 (—2)	1.804 ± 0.012 (—5)
Humerus	0.3160 ± 0.0034	0.3009 ± 0.0033 (—5)	0.3104 ± 0.0041 (—2)	0.3137 ± 0.0046 (—1)
Femur	0.7527 ± 0.0071	0.6978 ± 0.0088 (—7)	0.7112 ± 0.0088 (—6)	0.7206 ± 0.0104 (—4)
Organs with loss relatively less than that of body				
Testes	2.664 ± 0.053	2.276 ± 0.034 (—15)	2.096 ± 0.053 (—21)	2.262 ± 0.061 (—15)
Lungs	1.265 ± 0.033	0.940 ± 0.024 (—26)	0.983 ± 0.044 (—22)	0.983 ± 0.058 (—22)
Integument	36.92 ± 0.40	26.38 ± 0.18 (—29)	25.79 ± 0.24 (—30)	25.43 ± 0.45 (—31)
Epididymides	0.758 ± 0.031	0.530 ± 0.008 (—30)	0.518 ± 0.017 (—32)	0.568 ± 0.017 (—25)
Hypophysis	0.00784 ± 0.00018	0.00547 ± 0.00018 (—30)	0.00507 ± 0.00021 (—35)	0.00542 ± 0.00015 (—31)
Skeleton and musculature	121.71 ± 0.57	84.49 ± 0.52 (—31)	82.25 ± 0.57 (—32)	83.27 ± 0.70 (—32)
Kidneys	2.371 ± 0.053	1.614 ± 0.042 (—32)	1.750 ± 0.034 (—26)	1.578 ± 0.023 (—33)
Organs with loss nearly proportional to that of body				
Thyroid gland	0.03382 ± 0.00079	0.02261 ± 0.00082 (—33)	0.02083 ± 0.00102 (—38)	0.02111 ± 0.00120 (—38)
Heart	0.8574 ± 0.0115	0.5576 ± 0.0069 (—35)	0.5255 ± 0.0075 (—39)	0.5400 ± 0.0082 (—37)
Limb muscles	9.672 ± 0.092	6.282 ± 0.085 (—35)	5.798 ± 0.113 (—40)	5.935 ± 0.040 (—39)
Submaxillary gland	0.4628 ± 0.0074	0.2637 ± 0.0096 (—43)	0.2997 ± 0.0082 (—35)	0.2267 ± 0.0068 (—51)
Prostate	0.457 ± 0.026	0.254 ± 0.013 (—45)	0.243 ± 0.013 (—47)	0.293 ± 0.014 (—36)
Organs with loss relatively greater than that of body				
Intestines	3.552 ± 0.155	1.890 ± 0.058 (—47)	1.988 ± 0.075 (—44)	1.767 ± 0.047 (—50)
Spleen	0.460 ± 0.012	0.229 ± 0.010 (—50)	0.247 ± 0.057 (—46)	0.219 ± 0.010 (—52)
Pancreas	0.943 ± 0.035	0.466 ± 0.014 (—51)	0.547 ± 0.020 (—42)	0.428 ± 0.019 (—55)
Liver	11.50 ± 0.31	4.58 ± 0.16 (—60)	5.040 ± 0.092 (—56)	4.91 ± 0.24 (—57)
Gastro-intestinal contents	14.18 ± 0.51	4.25 ± 0.09 (—70)	6.45 ± 0.20 (—54)	4.92 ± 0.17 (—65)
Thymus	0.382 ± 0.022	0.0783 ± 0.0059 (—79)	0.0644 ± 0.0038 (—83)	0.0589 ± 0.0047 (—85)

The present results are in accordance with numerous observations in the literature, indicating that the spleen (like the lymphoid organs in general) suffers a relatively large loss in weight during inanition. Jackson [1] likewise found a loss of 51 per cent in splenic weight during acute inanition, but a decrease of only 29 per cent during chronic inanition.

Pancreas.—The loss of 55 per cent in the weight of the pancreas in the rats on the energy diet is but insignificantly higher than that (51 per cent) in the rats on tap-water. However, the loss of 42 per cent in the rats on the dietary accessories is significantly lower than that in either those on tap-water or those on the energy diet (with significance ratios of 3.3 and 4.3, respectively). There is consequently a definite indication of a specific influence in the accessory factors protecting against loss of weight in the pancreas.

Liver.—The loss of 57 per cent in hepatic weight in the rats on the energy diet is but insignificantly lower than that of 60 per cent in the rats on tap-water. The loss of 56 per cent in the rats on the dietary accessories is possibly significantly lower than that of the rats on tap-water (significance ratio, 2.5), but this can scarcely be attributed to the protective action of the accessory factors, since the result was not appreciably different in the rats on the energy diet with the accessories absent.

A marked loss of hepatic weight during inanition has likewise been observed by numerous investigators. Jackson [1] found a loss of 58 per cent during acute inanition, and of 43 per cent during chronic inanition, in rats with losses in body weight of 34 and 36 per cent, respectively.

Thymus.—The thymus shows its well known susceptibility to inanition with a loss of 79 per cent in the rats given tap-water. The somewhat greater decreases of 83 per cent in those fed the dietary accessories and of 85 per cent in the rats on the energy diet are of doubtful significance (with significance ratios of 2 and 2.6, respectively). In the earlier studies of Jackson,[1] no apparent decrease was found in the thymus of the adult rat during either acute or chronic inanition; but this was thought to be probably because the thymus in these animals had already undergone an age involution.

COMMENT

As was noted in connection with the various organs, the results of the present study for the most part agree with the earlier experiments of Jackson.[1] This is true also with respect to the investigation of Hayashi,[11] which unfortunately is available to us in abstract only. He reported that in acute and chronic inanition the organs of the white rat

11. Hayashi, Y.: Kyoto Ikadaigaku-Zasshi, July, 1924, vol. 21, no. 7; abstr., Jap. J. Zool. **1**:141, 1926.

fall into three groups: (1) those with weights decreasing more rapidly than the body, including the thymus, spleen and liver; (2) those decreasing parallel with the body, including the pancreas, parotid gland and submaxillary gland; (3) those decreasing less rapidly than the body, including the kidney, ovary, testis, hypophysis, thyroid gland and heart. The suprarenal gland did not lose weight, but often gained. Aside from the organs not included in the present study (ovary, parotid gland), it will be noted that Hayashi's classification is in agreement with our results, except for the pancreas and the thyroid gland, in which we obtained relatively greater losses in weight.

The effects of inanition on the weights of the various organs in the rat are also in general agreement with the scattered observations for other species (including man), although some differences appear. The literature on this subject has been reviewed at length by Jackson,[12] and therefore does not require further comment here. However, it should be emphasized that the results of inanition in adults (both man and animals) are in general not comparable with those in the young, in whom the effects are often strikingly different.

In the present work, relatively few differences are disclosed on comparing the group fed the dietary accessories with that given tap-water, on the one hand, and with that on the energy diet, on the other. This may be on account of the relatively short period of the experiment, which did not sufficiently exhaust the stored nutritional accessories to produce deficiency disorders in the rats on tap-water and on the energy diet. Similarly, the increased length of the inanition period during the tests with the dietary accessories and the energy diet was not sufficient to bring out some differences which might be expected from chronic inanition, in comparison with the more acute starvation in the rats on tap-water.

SUMMARY

Sixty well nourished male albino rats weighing 240 Gm. each were divided into four groups of fifteen each. The normal controls (group 1) were immediately killed and dissected. Test groups were placed on (2) tap-water only, (3) a diet deficient in calories but containing the normal requirements of protein, salts and vitamins ("dietary accessories"), and (4) a diet of starch and sugar ("energy diet") containing calories equivalent to those of the dietary accessories. All test groups were killed and examined when the loss in body weight reached 40 per cent.

12. Jackson, C. M.: The Effects of Inanition and Malnutrition upon Growth and Structure, Philadelphia, P. Blakiston's Son & Company, 1925; Arch. Path. **7**:1042, 1929; **8**:81 and 273, 1929; article on Inanition in the Cyclopedia of Medicine, Philadelphia, F. A. Davis Company, 1933, vol. 7 (in press).

The time required averaged 12.27 days for the rats on tap-water, 20.27 days for those on the dietary accessories and 17.33 days for those on the energy diet. The consumption of water was greatly increased in the rats fed the dietary accessories. In general, the external appearance was best in the rats on the energy diet, and no signs of specific deficiency disorders were observed. In all cases the body became emaciated, with very slight decrease in body length and practically unchanged tail length.

In the rats given tap-water only, the various organs and parts can be classified into four groups, according to their relative changes in weight. 1. The spinal cord, eyeballs, brain, humerus and femur remained nearly constant, while the stomach and suprarenal glands even gained slightly. 2. The testes, head, lungs, integument, epididymides, hypophysis, skeleton and musculature (combined) and kidneys lost weight, but relatively less than the body as a whole. 3. In the thyroid gland, heart, limb muscles, submaxillary gland and prostate (also in the visceral group as a whole) the loss was nearly proportional to that of the body. 4. The decreases in the intestines, spleen, pancreas, liver, gastro-intestinal contents and thymus (also in the "remainder") were relatively greater than that of the body.

The changes in the rats on the dietary accessories, in comparison with those in the rats on tap-water, are in most cases very similar. Significantly smaller losses in the pancreas and gastro-intestinal contents (and probably in the submaxillary gland and kidneys) may perhaps be ascribed to a protective influence of the dietary factors, since similar differences were not found in the group on the energy diet. On the other hand, somewhat greater losses in the weights of the stomach and testes occurred in the rats on the dietary accessories, but not in those on the energy diet. Finally, apparently greater losses in the thymus, limb muscle and (possibly) heart weights in both the group on the dietary accessories and that on the energy diet, in comparison with the group on tap-water, may perhaps be due to the shorter period of inanition in the latter group.

METASTASIZING CHONDROSARCOMA OF THE CORONOID PROCESS OF THE MANDIBLE

J. G. PASTERNACK, M.D.
Assistant Surgeon, United States Public Health Service
AND
R. D. LILLIE, M.D.
Surgeon, United States Public Health Service, National Institute of Health
WASHINGTON, D. C.
AND
R. A. JONES, M.D.
Surgeon (R), United States Public Health Service, United States Marine Hospital
SAN FRANCISCO

According to the literature, true enchondroma (not osteochondroma) arises only from bones of cartilaginous origin. Enchondroma of the skull is held to be a rarity. Occasional enchondromatous moles of the scalp have been observed that had no connection with the skull. Their origin remains unexplained. About the jaws, enchondromas are more often seen, but it must be remembered that the lower jaw has foci of cartilaginous origin, and that tumors of the upper jaw usually arise in connection with the wall of the maxillary antrum, which is a part of the cartilaginous primordial skull.

The tumor herein reported developed in the coronoid process of the mandible, a membrane bone, and produced extensive metastases of unusual size.

REPORT OF CASE

History.—J. A. C., a white man, aged 26, entered the United States Marine Hospital, San Francisco, on April 8, 1928, complaining of a painful swelling in front of the left ear. This he first noticed in January, 1928; he applied for treatment in May, 1928, but after roentgenologic studies had disclosed the presence of a tumor, he left the hospital untreated. On his return in April, 1929, an obvious deformity of the left side of the face existed. The patient complained of intermittent pain, which was slightly augmented by chewing hard food. The swelling had always been hard. There had been no salivary disturbance. At this time the patient had lost 20 pounds (9 Kg.) in weight in four months. He did not know of any cause for the appearance of the tumor except that five years previously he had fallen, striking his chin on the floor.

Examination disclosed the left tonsil flattened and the anterior faucial pillar deeply congested. Near the left mandibular articulation 1 inch (2.5 cm.) above the angle of the jaw a hard tumor was found, smaller than a golf ball and painful on pressure. It was of bony consistency, smooth and fixed. It was thought that the mass occupied the infratemporal fossa and was closely associated with or attached to the coronoid process of the mandible.

Roentgenograms disclosed a partly calcified mass about 2½ inches (6.27 cm.) in diameter in the left parotid region that had eroded the condyle and ramus of the left mandible. The destruction of the mandible appeared to be due to pressure rather than to malignant infiltration. There was probably some erosion also of the zygoma as well as of the maxilla. The calcification in the mass was irregular. The mandibular joint appeared normal.

Operation was performed on May 4, 1929. Arising from the left coronoid process of the mandible, a hard, elastic tumor was found. This filled the zygomatic fossa and the space between the ramus of the jaw and the mouth and cheek. It was the size of a baseball and definitely encapsulated. A cut section disclosed large cartilaginous areas and foci of calcification. Microscopic examination showed areas of spindle cells grading through myxomatous tissue into areas of

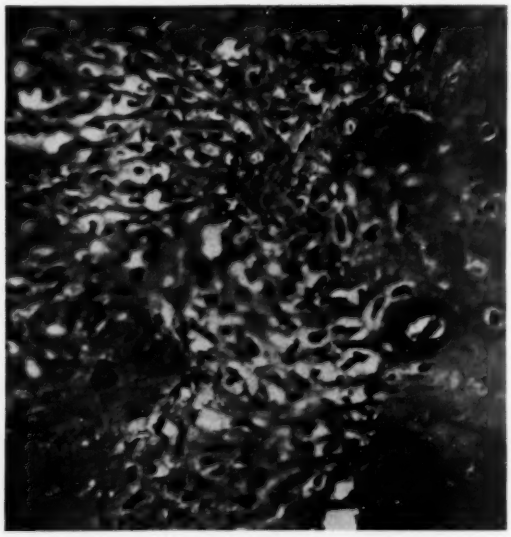

Fig. 1.—A microscopic section of the primary tumor.

cartilage. Cartilaginous matrix appeared predominant, although collagen fibers prevailed in the areas of spindle and stellate cells. The cellular areas contained numerous empty vascular spaces lined by endothelium.

The patient received local radium therapy while in the hospital. On Sept. 3, 1929, he was discharged apparently cured.

On March 27, 1931, he was readmitted with a recurrence of the tumor. An orange-sized mass was present at the old site. Marble-sized masses were present over the left occipital region, the right jaw, over the right shoulder and above the spine of the scapula. A mass the size of a small orange was present below the right costal margin. A mass thought to be the spleen was palpable in the left hypochondrium. The patient had lost some weight.

The red blood count was 5,120,000 and the hemoglobin 102 per cent; the white count was 12,000, with neutrophils 60 per cent, small mononuclears 35 per cent, transitionals 3 per cent and eosinophils 2 per cent; no abnormal forms were seen.

Roentgenograms showed no evidence of metastasis in the lungs.

A biopsy specimen of the tumor from the wall of the right side of the chest showed large blood-filled spaces lined by endothelium and marginated by compactly cellular mantles of small rounded and angular cells having scant cytoplasm and relatively large round or oval nuclei with small nucleoli and fine chromatin granules. Few mitoses were present. In areas, capillary proliferation was seen; elsewhere interlacing collagen fibrils were present, and the cells assumed spindle-shaped and stellate forms. The tumor was surrounded by a fibrous capsule.

The patient received short courses of roentgen therapy, which reduced the size of the tumors. His condition grew progressively worse, and the body came to autopsy on July 31, 1931.

Fig. 2.—The gross appearance of the cut surface of the kidney and the attached tumor; reduced one-half from actual size

Findings at Necropsy.—The body was extremely emaciated. There was little subcutaneous and omental fat. Metastatic nodules from 2 to 3 cm. in diameter were present in the right inguinal region, over the sixth right rib near the sternum and in the fifth intercostal space of the left side. On section, these nodules were hard, white, glistening and more or less homogeneous.

The right lung weighed 620 Gm. Adhesions were present at the base and on the lateral and posterior walls. The apex was scarred, and the parenchyma was emphysematous throughout. Tumor nodules 1 cm. in diameter were found in the upper and the lower lobe, respectively. Anthracosis was marked, and the glands at the hilus were enlarged.

The left lung weighed 760 Gm. It was bound down to the diaphragm and to the wall of the chest by dense adhesions. There were no palpable nodules. A tumor nodule 1.5 cm in diameter was present in the second intercostal space

The pericardium contained 250 cc. of clear straw-colored fluid. The heart weighed 250 Gm. and showed no abnormalities.

The liver weighed 1,450 Gm. Palpation disclosed a nodule just below the anterior surface of the right lobe. Section showed a dense, white nodule 1 cm. in diameter.

The spleen weighed 170 Gm. It was moderately congested.

The mesentery was studded with numerous firm, hard, nodular masses varying in size from 0.5 to 5 cm. in diameter.

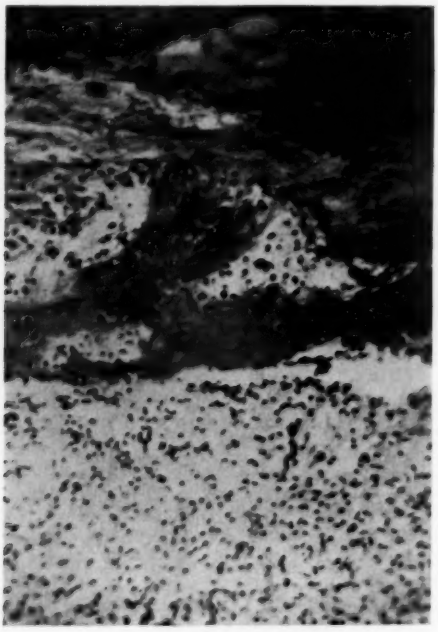

Fig. 3.—A microscopic section from the tumor around the kidney.

In the pelvis at the bifurcation of the aorta there was a group of firm, enlarged glands that presented the appearance of the tumor tissue seen elsewhere. The prostate was small and firm.

The right kidney weighed 390 Gm. and was about 20 cm. in length. There was a nodular mass the size of a large orange at the upper pole, and at the lower there were several pedunculated masses about 5 cm. in diameter. On section, the kidney was intact and sharply demarcated from the tumors. The renal capsule stripped off easily. A moderate amount of peripelvic fat was present. Section of the mass at the upper pole showed a white, firm, more or less glistening mass apparently made up of confluent smaller nodules, some of which were readily identified as cartilage and calcified plaques.

The left kidney weighed 1,090 Gm. It produced more or less visceral displacement. The spleen was pushed down. Adhesions to the stomach and diaphragm were present.

The kidney and tumor were 18 cm. long, and the tumor was 10.5 cm. wide. The tumor formed a bulky pyriform mass that surmounted the upper pole of the kidney. It was encapsulated and sharply demarcated from the renal substance. Cut section disclosed a more or less lobulated mass consisting of circumscribed white, hyaline areas, areas of calcification and narrow, ramifying zones of tendinous tissue. The cortex at the upper pole of the kidney was compressed but not obviously infiltrated.

Microscopic Observations.—The metastatic tumor nodules were largely cartilaginous, but in areas consisted of myxomatous or cellular mesenchymatoid tissue traversed by large blood-filled, endothelium-lined vascular spaces.

The splenic pulp cords were hypertrophied and often contained collagen fibrils; the reticulum cells were flattened; the sinuses were widened and unusually distinct. The malpighian corpuscles were small and hypoplastic.

Several sections of the lungs showed no tumor growth in the parenchyma or in the vascular channels. There were areas of nodular consolidation in which fibrinopurulent exudate and desquamated epithelial cells made up the alveolar contents. The pleura was covered by necrosing fibrinopurulent exudate.

The liver cells were large and finely reticulogranular. Slight interlobular fibrosis was seen. A cellular tumor nodule was composed of large stellate and closely packed round cells having large round, oval and elongated leptochromatic nuclei, showing more or less hyperchromatism and distinct nucleoli; it showed a closely packed mesenchymelike arrangement grading into areas of cellular hyaline cartilage. In areas, the cartilage showed a cancellous configuration containing vascular spaces, osteoclasts and lamellae of osteoid and osseous tissue. Scattered areas of nuclear pyknosis grading into coagulative and hemorrhagic necrosis were found in the tumor.

The tumor attached to the kidney consisted of irregularly commingled areas of dense mesenchymatoid tissue composed of small, round and spindle-shaped cells, myxomatous tissue, hyaline cartilage, fibrous tissue, osteoid tissue and partly calcified cancellous bone.

One small myxochondromatous nodule was found in the cortex of the right kidney.

The kidneys themselves showed only slight cloudy swelling.

The suprarenal glands could not be found in the dissected specimens of kidney and tumor.

The heart contained areas of interstitial fibrosis.

The prostate showed slight interstitial fibrosis.

COMMENT

Koller reported that Hanau had the opportunity of studying a tumor of the coronoid process of the mandible. This tumor was a combination of chondroma and osteochondroma with partial transition to chondrosarcoma. It is generally held that chondromas of bone arise from cartilaginous rests that remain from the process of endochondral ossification, but membrane bones do not arise in this manner and hence cannot retain such remnants. With the exception of the angle, a portion of the ramus and a small part near the symphysis, which are of cartilaginous origin, the mandible is said to be formed by the intramembranous method of ossification.

On this basis, tumors of the coronoid process would arise in an area usually not preformed in cartilage. That cartilage can develop from the periosteum of membrane bone even as it can from the periosteum of bone developed in cartilage is no longer in doubt. According to Koller, cartilage was formed in experimentally produced fracture callus of membrane bones. However, the development of an enchondroma or of a chondrosarcoma from normal periosteum is problematic.

It is generally held, and it is stated in current textbooks, that the coronoid process of the mandible is membranous in origin. But Koller stated that Hanau found the coronoid process of a 24 to 25 cm. fetus composed of hyaline cartilage entirely distinct from Meckel's cartilage, and Masquelin reported the presence of cartilage in the coronoid process of 7.5 and 9.5 cm. embryos. From this it is evident that secondary cartilaginous anlagen in the coronoid process occur more frequently than is commonly supposed. Consequently, tumors arising from the coronoid process arise in areas of disturbed ossification where embryonal cartilaginous rests are present. To no tumor does Conheim's hypothesis apply so well as to cartilaginous tumors of the type described.

SUMMARY

A case of chondrosarcoma of the coronoid process of the mandible is reported. There were extensive generalized metastases and especially large metastatic masses around the kidneys.

Derivation from a primitive cartilaginous anlage is suggested, as it has been shown that the coronoid process is, at least at times, preformed in cartilage distinct from Meckel's cartilage.

MULTILOCULAR CYSTIC LYMPHANGIOMA OF THE SPLEEN

CHARLES W. McLAUGHLIN, M.D.
Surgical Clinic of Dr. E. L. Eliason
AND
JAMES S. P. BECK, M.D.
Department of Pathology of the William Pepper Laboratory of Clinical Medicine
PHILADELPHIA

The purpose is to present an interesting and unusual lesion in the spleen of a man aged 42. It was a large eccentric multilocular cyst that caused pronounced splenic enlargement. It was not associated with any lesions in the other abdominal organs. These pages also include a brief summary of the clinical aspects of such lesions.

Nonparasitic cysts of the spleen are infrequent. Andral [1] in 1829 reported the first case, one of the three cases of dermoid cysts of the spleen on record. Fowler [2] in 1921 collected ninety-three cases of nonparasitic cysts of the spleen, of which sixty-five were of the large type. The latter number was increased to seventy-three by Frank [3] in 1927. Since that time Novak,[4] Dabrzaniecki,[1] Bancroft and Jessup,[5] Brandberg [6] and Benton [7] have reported cases. The case that we report adds another to this small series. It has been classified as lymphangioma, a small, interesting subgroup. The incidence of primary tumors of the spleen is low. Krumbhaar [8] found only six cases in 6,500 autopsies. One was a lymphangioma.

Because of the rarity of lymphangioma of the spleen the data on this case are given in full.

REPORT OF CASE

C. R., a man aged 42, was admitted to the service of Dr. C. C. Wolferth on April 23, 1932, complaining of discomfort in the left upper quadrant of the abdomen. The past history was not remarkable, except for a mild glycosuria nine years before and a digestive disturbance simulating peptic ulcer one year later, both of which cleared up on dietary treatment. The discomfort was first noted

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From the Hospital of the University of Pennsylvania.

1. Andral, quoted by Dabrzaniecki, W.: Ann. Surg. **92**:67, 1930.
2. Fowler, R. H.: Ann. Surg. **74**:20, 1921.
3. Frank, L. W.: Ann. Surg. **85**:360, 1927.
4. Novak, E.: Surg., Gynec. & Obst. **45**:586, 1927.
5. Bancroft, F. W., and Jessup, D.: S. Clin. North America **8**:1015, 1928.
6. Brandberg, R.: Acta chir. Scandinav. **63**:346, 1928.
7. Benton, R. W.: J. A. M. A. **99**:1674, 1932.
8. Krumbhaar, E. B.: Ann. Clin. Med. **5**:833, 1927.

one year before the present admission to the hospital. At that time an x-ray picture showed a notch in the splenic flexure of the colon, supposedly due to pressure from the spleen. All symptoms disappeared until February, 1932, when the patient again became aware of abdominal discomfort and observed that his abdomen was enlarging. Recently fresh blood was noted at the end of defecation. He was sallow, but not icteric. The cardiovascular system was normal. A palpable, slightly tender mass was noted in the left upper quadrant of the abdomen, moving with respiration. It was thought to be the spleen, although a notch could not be felt. There was no fluctuation. Just below this mass was a body, the size and shape of the left kidney.

The red blood cells numbered 4,650,000 per cubic millimeter. The hemoglobin content (Sahli) was 90 per cent (15 Gm.). The white blood cells numbered 6,500 per cubic millimeter, with neutrophils 74 per cent, large mononuclears 2 per cent, lymphocytes 22 per cent, and eosinophils 2 per cent. The platelets numbered 270,000 per cubic millimeter. The clotting time was normal. The results of a fragility test were: beginning hemolysis, 0.450 per cent; complete hemolysis, 0.300 per cent. The Wassermann and Kahn tests were negative. The urine was normal. The feces were normal (except for blood). The renal function was normal (both sides). The icterus index was 6 units. The van den Bergh direct test was negative; the indirect, positive, 2 units. A test of liver function by means of bromsulphalein showed 30 per cent remaining in the blood stream in five minutes and 5 per cent in thirty minutes. The roentgen report was: a notch in the splenic flexure of the colon; the left side of the diaphragm slightly elevated; the left kidney (uroselectan) displaced downward and the left ureter kinked.

An early case of Banti's disease, splenic anemia and a neoplasm, including adenoma of the suprarenal gland and dermoid cyst, were considered. At operation, a large cystic spleen with few adhesions was removed. No other changes were found. The patient did well postoperatively. The platelet count rose following operation to 560,000 on the sixth day and to 768,000 on the tenth, returning to 544,000 on the fourteenth day. The white blood cells numbered 15,000 on the third day, but decreased to 9,100 on the ninth. The patient was discharged on the nineteenth day.

The Spleen.—The organ weighed 1,620 Gm. and measured 18 by 14 by 12 cm. It was roughly ovoid, tense and fluctuant, except in the lower part where a portion of the spleen, corresponding roughly to about one fifth of the specimen (about one half of a normal spleen), had a generally normal appearance. The capsule was finely wrinkled over the lower, nearly normal part. In general, the organ was livid, but over the external face of the tense portion were large pale areas where the wall was thin. Except for a few fibrous tags in this region, the capsular surface was smooth, though elevated by two groups of small subcapsular serous cysts. The larger group formed a row of closely set single and multilocular cysts that fringed the inferior border. They varied in size, but did not exceed 1 cm. across. The other group, consisting of three similar cysts, was located in the capsule of the tense portion of the spleen at the margin of a shallow groove. One of them was partly filled with blood.

The method of examination of the spleen was as follows: While still warm, it was perfused with warm Locke's solution through the splenic veins. The pressure was carefully watched to prevent overdistention of the organ or rupture of the vessels. When the blood vessels were cleansed, Helly's fixing fluid was substituted for Locke's solution, and the perfusion was continued until all parts of the organ were colored with the chromates of the fixative. At this point the perfusion was stopped, and the vessels were ligated. The specimen was then immersed in Helly's

fluid, where it remained until the next day. In sectioning the spleen, one deep longitudinal incision was made over the thinnest wall of the cyst and carried down almost to the hilus. Following inspection and the securing of blocks for paraffin and frozen sections, the organ was carefully dissected in order that the vascular arrangement in the cyst wall might be inspected.

The eccentric enlargement was a large cyst filled with thick, greenish-yellow fluid (color probably altered by Helly's fluid). The cyst, limited by a fibrous coat from 1 to 2 mm. thick, occupied the upper four fifths of the spleen. The thinnest

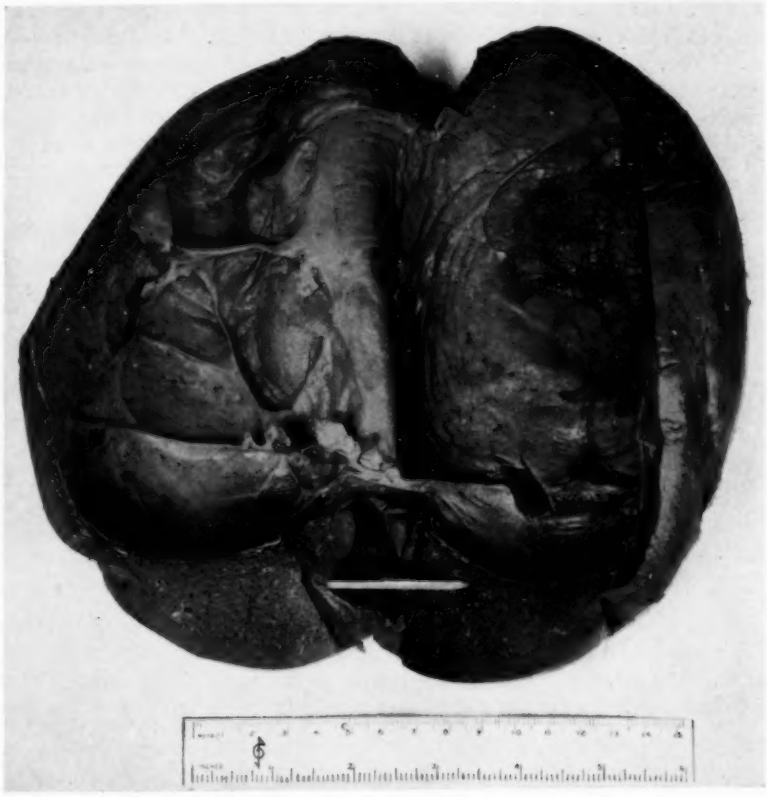


Fig. 1.—A large multilocular cyst of the spleen. The position and appearance of the cyst and its septums are shown. A leaflike fragment of a septum is pressed against the wall of the cyst. A thick deposit of crystals is seen in the dependent part of the main cavity.

walls were in the upper polar and lateral regions of the organ. In places corresponding to the pale areas noted in the gross inspection, the walls were composed of only the fibrous coat of the cyst and the capsule of the spleen. The walls thickened gradually as they converged at the hilus, owing to an increasing amount of pulp that separated the cyst from the capsule. This pulp was compressed, the gross structures of it appearing in concentric layers around the cyst. The lamination was absent in the pulp of the lower one fifth of the spleen. The cyst had several incomplete septums which divided the cavity into a main cyst, 12 cm. across, and

five or six other smaller ones (fig. 1). The septums projected from the medial wall and did not completely traverse the internal surface. For the most part they were low, the free margins being smooth and rounded, but in some places the septums were continued into thin greenish, brittle sheets that extended some distance into the cavity. The general internal surface was grayish white and, aside from the septums, was smooth, though elevated in the medial wall by a trabecular pattern of low, flattened ridges and white lines. These were the remains of splenic blood vessels and trabeculae, and they passed over into the pulp without a zone of demarcation. There was a thick deposit of cholesterol crystals in the dependent part of the main cyst. In one area was a group of minute oval depressions, about which was a fine deposit of brownish granular material that could be scraped away. Under magnification, this was found to be composed of refractile granular débris, cholesterol crystals, a few red blood cells, lymphocytes and an occasional macrophage. There were no communications with the flattened vessels in the outer part of the cyst coat. A few fine petechiae were in the lining membrane.

Fig. 2.—A drawing of a part of the dissection. The compressed splenic pulp of the median wall of the cyst separates into layers, each of which is supplied by arteries. The relation of the flattened veins to the arteries is shown. The third and deepest layer is very thin and adheres closely to the fibrous coat of the cyst.

The Dissected Spleen.—The fat and fascia around the hilus were normal. The hilus was about 12 cm. long, and four main arteries were found. The branches that supplied the normal portion of the spleen were slightly tortuous; those supplying the cyst were stretched. The splenic veins were thin-walled, and the intima was smooth and shining. To follow these vessels into the substance of the spleen, the capsule was incised along the hilus and reflected. It separated easily from the compressed pulp, but was difficult to separate from the fibrous coat of the cyst in the thinnest portions of the cyst wall. In following the vessels it was observed that the pulp yielded readily to blunt dissection and tended to separate itself into definite layers (fig. 2).

This is an interesting observation, and it has not been previously described, as we found in our review of splenic cysts. It is recalled that anatomically the spleen is made up of units which are not separated by connective tissue as in other organs, but are recognized only as vascular structures. Each main arterial branch that enters the splenic substance at the hilus supplies a definite portion without signifi-

cant anastomosis with other large branches. It occupies the central portion of this unit of splenic tissue, and its branches enter the small units, or lobules, as central arteries, exhausting themselves therein. The veins occupy a peripheral position relative to these units. From this it can be visualized that, with progressive compression by a constantly enlarging cyst and atrophy, these units would not only be displaced in advance of the cyst, but would be flattened into layers between the cyst and the capsule. The arteries by this mechanism would approximate the veins in each large unit and become more closely related to them.

Exactly this picture was found when the dissection was complete. In the medial wall were three distinct layers, three arterial branches, and three flattened veins. A vein lay beside each artery; the two, in a common connective tissue network, formed the principal vessels in each of the three layers that separated out during dissection. The vessels of each layer occupied its central portion. The deepest was thinnest and lay on the fibrous coat of the cyst and adjacent to the trabecular pattern of the internal surface. These layers thinned out gradually to become imperceptible in the opposite wall where the fibrous coat of the cyst was against the capsule. The arteries were 1 mm. in diameter as they penetrated the splenic capsule at the hilus, and tapered normally in their distribution. In no place was there observed a focal change or an abrupt termination of a vessel. The veins were transparent and smooth. There were no locular dilatations. No structures were interpreted as dilated lymph channels.

Microscopic Description.—The Arteries: Several sections of the arteries and their branches revealed an irregular thickening of the intima by hyaline tissue with an occasional subjacent interruption of the elastica. The central arteries here and there had eccentric intimal thickenings, which, in some instances, stained with sudan III. In a few the lumens were almost occluded. The media and adventitia were not remarkable.

The Cyst Wall: The fibrous coat of the cyst was about 1 mm. thick, and an occasional area was lined with flat cells. The inner part was made of hyaline bands, and the outer part was composed of compressed remains of blood vessels, trabeculae and pulp. The pulp between this coat and the capsule of the spleen was laminated. The cords and sinuses appeared as alternating bands and elongated spaces parallel to the layers formed by the larger structures. The malpighian bodies were small and oval. The lymphocytes were diminished, making prominent the connective and reticular tissues, especially in the cords. There was some extravasation of blood near the fibrous coat of the cyst. The capsule was thickened by fibrous tissue. The septums, from 1 to 2 mm. thick near the bases, were composed of three strata: two thin superficial layers of hyaline tissue and a middle portion containing a deposit of calcific material.

The Subcapsular Cysts: The smallest of the superficial cysts at the inferior border were single and averaged 1 mm. in diameter. The larger ones were multilocular and were about 1 cm. across. They were under the capsule, separated from it by a narrow zone of compressed splenic tissue. The septums of the larger cysts were trabeculae and strands of splenic tissue. The lining was a single layer of flat cells; some were loosened and vacuolated, and lay singly and in groups in an eosin-staining precipitate that filled the cysts. An occasional hemorrhage was seen. The contents of one of the cysts from the tense portion of the spleen was half precipitate and half blood. In one section, under the capsule, were several cavernous spaces lined with endothelium. There was some condensation of the adjacent tissues about the more prominent small cysts. The portion of the spleen appearing nearly normal was not remarkable, except for some crowding of lymphocytes beneath the capsule, which was slightly thickened with collagen fibers.

COMMENT

The classification of nonparasitic cysts generally accepted is that proposed by Fowler,[9] who grouped them into traumatic, inflammatory, degenerative, dilatation and neoplastic cysts. Traumatic cysts occur in two forms: large unilocular cysts, such as encysted hematoma, that have hemorrhagic or serous content, and are by far the most common variety; and multiple small cysts, superficial or deep, which arise from peritoneal inclusions, a rare type. The inflammatory cysts arise from tuberculous foci or, in perisplenitis (due to malaria, leishmaniasis, etc.), entrapped groups of mesothelial cells of the capsule as a result of inflammatory swelling. The degenerative cysts are solitary and large, arising secondarily in infarcted areas from arterial change or occlusion by emboli with consequent necrosis of the pulp. Dilatation cysts result from ectasis of splenic sinuses (polycystic disease—Coenen-Fowler). They are multiple and fused, riddling the spleen. The neoplastic types include lymphangioma, hemangioma and rarer conditions such as dermoid cysts.

Splenic cysts occur most frequently in persons between the ages of 20 and 40. Women in the child-bearing age are affected in 65 per cent of the cases. Frank [10] found a history of trauma in 22 per cent of his series. No statistical reports have been made regarding the incidence of infection. The differential criterion of dilatation and neoplastic cysts (Fowler) is as yet unknown. Fowler [2] in a series of ninety nonparasitic cysts found twelve classified as lymphangioma. Developmental errors have been considered as a cause of some of these cysts.

Our specimen is a large multilocular cyst associated with two groups of small cysts in the subcapsular region of the spleen. In the history there is nothing pointing to traumatism and infection. The examination failed to demonstrate evidence on which to assign the cyst to the inflammatory or to the degenerative group. There was perisplenitis at one time, as evidenced by the adhesions on the capsule, but the groups of small cysts were in other areas, and microscopically they were more like cystic lymph spaces than cysts due to associated inflammation. The adhesions of this specimen are regarded as resulting from the swelling by the large cyst and not related to the cause of the small cysts.

This variety of small cyst seems to be a fairly common finding in sections. As they do not attain to a large size, they are of no clinical importance. Their mode of origin has been fully discussed by Brandberg,[6] who summarized the consensus by stating that the foregoing pathologic-anatomic scheme (peritoneal inclusions, small ruptures of

9. Fowler, R. H.: Ann. Surg. **80**:58, 1924.
10. Frank, L. W.: South. M. J. **23**:212, 1930.

the capsule by trauma, or inflammatory swelling) is not generally accepted, for there are numerous authors (Alfejew, Aschoff, Fink, Kuhne, M. B. Schmidt [11]) who consider that the cyst formations in question are as a rule caused by dilatation of lymphatic vessels. With this assumption, it is thought, the possibility ought to be admitted that these small multiple cysts may develop into larger cysts that possess surgical interest. Brandberg [6] supported Howald and others in stating that the association of small cysts, though microscopic in size, with a large cyst is evidence that the lesion represents lymphangioma. He further stated that trabeculation, bloody fluid in the cyst and pigment in the wall of the cyst do not exclude a lymphatic cyst, and that an endothelial lining is not of paramount importance. It is then assumed that all cysts, small and large, in this syndrome are of lymphatic origin. That this is always true may be questioned. Small cysts may be found in splenomegaly, corresponding in size and boundary to the small subcapsular anatomic lobules originally described by Franklin Mall.[12] These lobules are poorly outlined and subdivided by trabeculae. A cystic change in one of these units would present a multilocular appearance, the loculi being outlined with trabeculae.

Most of the lymphangiomas of the body are of the cavernous type, and the association of cavernous spaces with cysts is frequent in these neoplasms. Passive dilatation is given as a cause of lymphatic cysts, and while lymph stasis doubtless influences the course of many lymphangiomas, it is well known that edema and varicosities are by far the most frequent results of lymph channel obstruction.[13] Ribbert [14] argued that any marked pressure would inhibit the growth of endothelium, and he assumed that lymphatic and other cystic tumors develop as a result of a process of growth located in the walls which steadily enlarges the cysts and prevents the passive accumulation of fluids. Applying the principles of hydraulics to these channels where a flow is established, one must assume that there is a diminishing gradient of pressure of fluid from the areas where the lymph is formed to the point where the lymph finally empties into the great venous system. Suppose an obstruction is placed somewhere in the course of the channel and completely blocks the flow of lymph; the adjustment of pressures is such that the increase in the obstructed channel cannot exceed that in the

11. These names are quoted from Brandberg,[6] whose bibliography of this subject is complete up to 1928. Since then the literature on the subject has consisted mostly of case reports.

12. Mall, F. P.: Bull. Johns Hopkins Hosp. **9**:218, 1898.

13. Bradbury, Samuel: Nelson Loose-Leaf Living Medicine, New York, Thomas Nelson & Sons, 1927, vol. 4, chap. 8.

14. Ribbert, quoted from Ewing, James: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1928, p. 252.

areas of origin. The pressure rises in this channel only to a point equal to that in the areas of origin of the lymph, namely, the lymphatic capillaries and tissue spaces; hence the widening is no greater than the manifestation of edema in any complete mechanical blockage widespread enough to impede the flow of lymph through anastomotic and collateral branches. The effect of pressure is slightly different in the event that a small channel empties into a large, blind cavity. It follows Pascal's law which states that liquids transmit in all directions and without diminution any pressure that is applied to them. The principle is applied to the hydraulic press or ram. The force exerted on the wall of such a cavity is to the force in the small channel as the area of cross-section of the cavity is to that of the channel. A fluid pressure of 1 cm. of water in a channel 1 sq. mm. in cross-section is transmitted as 100 cm. of pressure in a water-filled cavity 100 sq. mm. in cross-section. It is possible for fluid in minute afferent channels to exert remarkable pressures in a fluid-filled cavity without the effect of excessive pressure on the channels themselves. Also, according to Bernoulli's theorem, increased fluid pressure in a widened portion of an unobstructed channel is to be expected, and this pressure will continue to increase as the cross-section of the widened part of the channel is increased. The application of known physical principles (hydraulic and osmotic) to pathologic changes has been too much overlooked. Such data specifically applicable in pathology have not accumulated and have to be taken from texts on physics and hydraulics. The aforementioned principle under suitable conditions can account for much pressure in cysts and other cavities where there is no obstruction to the flow of fluid, causing them to enlarge by mechanical dilatation without an associated edema.

The proliferative process in lymphangioma, theoretically, can vary from simple hyperplasia to malignancy. If the rate of proliferation keeps in advance of the intracystic fluid pressure (Ribbert), budding proliferative areas and multiple cyst formations are expected to be seen microscopically. This picture has been described (Brandberg and others) and is usually associated with numerous small cysts and cavernous spaces. Metastatic foci are expected to develop. On the other hand, if the rate of increase in pressure within the cyst exceeds the proliferative rate, it is logical to expect the results of excessive pressure. These are flattening of lining cells, inhibition of growth (Ribbert) and finally degeneration. This picture is common and the cysts are usually of the large type. Within the two extremes given here many variations are possible.

There is little etiologic information to be gained from a study of the fibrous coat of a cyst. Owing to the pressure of the cysts there

arises a thickening of adjacent tissues, and around the larger cysts a firm wall of connective tissue is formed in the course of time. This wall blends with the surrounding reticular tissue of which it is formed, and in this way all cysts become embedded in the splenic tissue irrespective of their origin.[6] The trabecular pattern of the internal surface of the cyst is informative, but not distinctive of lymphatic cysts. Since it is composed of trabeculae and blood vessels of the pulp which have become a part of the cystic coat, it is to be found in that part of the coat that has advanced against the pulp. If the cyst arose in the subcapsular region and obtained a large size, the trabecular pattern would be on the side next to the pulp, and the side against the capsule where the cyst arose would be smooth. A confirmation of this explanation is shown in the dissected specimen reported here. The septums in our specimen were definite, but showed an extreme degree of degeneration. They indicated that the large cyst was formed by the fusion, degeneration, rupture and retraction of the walls of the adjacent cysts. If time had been allowed, doubtless the septums would have disappeared beyond the point of recognition, and the cyst would have been the large solitary type.

From the discussion and evidence presented it is believed that the cyst under investigation arose originally as a lymphangioma in the subcapsular or capsular region where the lymphatics of the spleen are located. It increased in size, becoming very tense, from the original lymphangiomatous state by a possible combination of factors not usually considered in pathology—namely, those concerned in hydrodynamics and osmosis. During this stage degeneration from excessive pressure overcame and suppressed the original proliferative state, so that the final picture was a cyst with definite degeneration in the walls. The small cysts found beneath the capsule are interpreted as a part of the lymphangiomatous lesion. In this opinion we are in harmony with Brandberg and others who believe that a large cyst associated with small cysts is evidence that the lesions are lymphangiomatous.

SYMPTOMATOLOGY, DIAGNOSIS, TREATMENT AND PROGNOSIS

The symptoms are not suggestive of the lesion. Discomfort in the left upper quadrant of the abdomen and abdominal enlargement, varying in degree, may be present. Dyspnea and gastric and colonic disturbances may be observed. Melena may occur from obstructive venous congestion. Pain in the left shoulder was observed in Novak's case. Urinary and menstrual disturbances are not infrequent. Ascites rarely occurs. Although the cyst may be very large, fluctuation is not commonly elicited. A friction rub may be audible, secondary to perisplenitis.

Dabrzaniecki [1] stated that no recorded case was diagnosed before operation. The essential findings are usually limited to a mass in the left upper quadrant of the abdomen, occupying the position of the spleen. The usual causes of splenomegaly may in the majority of cases be excluded by the history and by examination of the blood. If fluctuation is present, retroperitoneal, mesenteric, pancreatic, renal and ovarian cysts must be considered. A pyelogram and an x-ray picture of the gastro-intestinal region often assist in the diagnosis. In our case, a notch in the splenic flexure of the colon, elevation of the left side of the diaphragm and displacement of the left kidney were present. Positive specific complement-fixation tests may confirm the suspicion of an ecchinococcic lesion. Splenic puncture is occasionally practiced, but should be considered a dangerous procedure.

Splenectomy is the method of treatment in all cases except those in which perisplenitis and adhesions make this procedure impossible. Pean in 1867 did the first splenectomy for splenic cyst (Fowler [2]). Fowler [9] was able to collect thirty-six cases treated by splenectomy up to 1924, with a 2 per cent mortality. Marsupialization has been suggested in those cases in which splenectomy is impossible.

Prognosis is uniformly good if the cyst arose on a traumatic basis and if it is not of the angiomatous group. In cases in which angiectatic lesions occur, however, the prognosis should be guarded. Such lesions may occur in the liver and lungs, as well as in the spleen. Brandberg and others reported cases in which, after successful splenectomy, cystic lesions developed in the liver with a fatal outcome.

SUMMARY

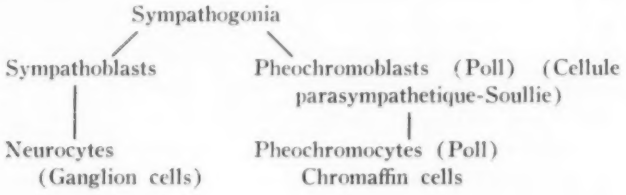
A large nonparasitic cyst of the spleen is described, which was treated successfully by splenectomy. The history was negative for trauma, and there were no demonstrable foci of infection. Clinically there was no evidence of arterial disease. The intrasplenic vessels, inspected by dissection, were found to have an abnormal relationship secondary to the cystic enlargement. Microscopically, arteriosclerotic changes, commonly seen in grossly normal spleens, were found. A careful gross and microscopic study of the specimen was made, and multilocular cystic lymphangioma of the spleen was diagnosed. It is shown that a consideration of the principles of hydraulics in connection with the rate of proliferation simplifies the problem of the pathogenesis of cystic tumors.

A SUPRARENALIN-PRODUCING PHEOCHROMOCYTOMA OF THE SUPRARENAL GLAND

REPORT OF A CASE

FORD K. HICK, M.D.
CHICAGO

The nomenclature of the tumors of the suprarenal medulla is best understood by following the schema of Wiesel showing the origin of the secretory cells, as contrasted with the ganglion cells, in common from an ectodermal stem cell, the sympathogonium, or *Sympathicus-bildungzelle* (Kohn).



A tumor may show any of these cell types, and at times two or more. The immature types of cells, the sympathogonia, are seen in the neuroblastomas of children, formerly described as sarcomas. The tumors consisting of sympathoblasts can scarcely be distinguished from those consisting of pheochromoblasts, since there is no microscopic differentiation between these cells. They differ only in potentiality. King [1] recently reported a unique case of a metastasizing tumor of the suprarenal medulla which took the typical brown color after fixation in a solution of chrome salts. This neoplasm had in its metastases mature pheochromocytes and, in addition, smaller, less regular cells, with darker-staining nuclei, clear cytoplasm and marked evidence of proliferation. He called it a malignant pheochromocytoma. It apparently contained both pheochromocytes and pheochromoblasts. (Bonnamour's [2] case of metastasizing pheochromocytoma is not convincing.) Paul [3] described the finding of both ganglion and pheochrome cells in a tumor encountered in the suprarenal gland of a man dead from cirrhosis of the liver. The mature ganglion cells with their processes stained yellow with van

From the Department of Pathology, Cook County Hospital (Dr. R. H. Jaffé, Director) and the Department of Medicine, University of Illinois.

1. King, E. J.: J. Path. & Bact. **34**:447, 1931.
2. Bonnamour, S.; Doubrow, and Montegue: Ann. d'anat. path. **4**:141, 1927.
3. Paul, F.: Virchows Arch. f. path. Anat. **282**:256, 1930.

Gieson's stain and a deep blue with Holzer's stain. In addition, there were anaplastic ganglion cells which certainly were malignant. The pheochromocytes took the brown color well after fixation in Zenker's solution. These are the only such mixed tumors described in the literature, but they serve to illustrate the clear identity of the cell types, and constitute proof of the accuracy of Wiesel's classification. Most pheochrome tissue contains a few neurocytes or sympathoblasts (Wiesel [4]), but the resemblance of the latter to lymphocytes is so close that there the statement is not beyond doubt.

PHEOCHROMOCYTOMA

True pheochromocytoma is among the rarest of tumors. The earliest cases were those of Berdez [5] and of Manasse.[6] Raben,[7] in an excellent summary of the literature, stated that only thirty instances of this tumor had been reported prior to 1929. This number included the proved chromaffin tumors arising anywhere in the paraganglions or in the carotid body. Pick suggested the name paraganglioma for such a tumor arising in the paraganglions, reserving for tumors of the suprarenal medulla the name pheochromocytoma, and Masson [8] refused to accept any tumor as a pheochromocytoma that arose outside the suprarenal gland. The way in which tumors come from the same stem cell in different sites makes the distinction somewhat artificial. Normally small islands of chromaffin cells may be found in any part of the sympathetic ganglion chain.

At present the cases of pheochromocytoma of the suprarenal gland in the literature total forty-two, the case that I shall describe being the forty-third. The cases appearing since Raben's review are those of Wichels and Biebl,[9] Vaquez et al.,[10] Shipley,[11] Labbé et al.,[12] Barker,[13] Paul [3] and King.[1] The criteria used in making the selection of cases could not be limited to the chromaffin reaction. That reaction disappears a few hours after death, and in not a few cases was gone before the postmortem examination could be made. Particularly is this true with respect to the French reports. The gross and histologic descriptions of such tumors in appropriate positions are characteristic.

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4. Wiesel, Josef: Anat. Hefte **19**:481, 1902.
5. Berdez: Arch. de méd. expér. et d'anat. path. **4**:414, 1892.
6. Manasse, P.: Virchows Arch. f. path. Anat. **133**:391, 1893.
7. Raben, C. B.: Arch. Path. **7**:228, 1929.
8. Masson, P.: Tumeurs, Paris, A. Maloine et fils, 1923, p. 546.
9. Wichels, P., and Biebl, M.: München. med. Wchnschr. **75**:656, 1928.
10. Vaquez, H., and others: Presse méd. **37**:169, 1929.
11. Shipley, A. M.: Ann. Surg. **90**:742, 1929.
12. Labbé, M.; Azérad, E., and Violle, P. L.: Bull. et mém. Soc. méd. d. hôp. de Paris **53**:952, 1929.
13. Barker, L. F.: M. Clin. North America **14**:265, 1930.

Pheochromocytoma in a Woman Dead from a Ruptured Aneurysm of the Anterior Communicating Artery.

History.—S. A., an Italian woman of 64 years, was brought into the hospital on May 11, 1932, in a stupor and died in a few hours. Her son said that she had been as well as usual until that morning when he found her unconscious on the floor. For the last few weeks she had slept much of the time during the day. On questioning, the family thought she had vomited a few weeks before and may have had convulsions then.

She was an obese woman with labored respirations and slight cyanosis, and was in a state of coma from which she could not be aroused. The respirations were 30 per minute; the pulse rate was 76, and the blood pressure 180 systolic and 105 diastolic. The pupils were unequal and fixed to light. The chest was resonant throughout, but coarse rhonchi obscured other findings. The heart was enlarged to the anterior axillary line on percussion. The tones were regular but distant. There were no murmurs. The abdomen was rounded and soft. No masses were felt. The extremities showed no edema. No reflexes could be elicited. The urine contained albumin, casts and red cells. The clinical diagnosis was: cardiovascular-renal disease with a cerebral accident, probably a hemorrhage. The autopsy was performed ten hours after death.

Essential Anatomic Findings.—There was a severe subarachnoid hemorrhage particularly about the cisterna magna. The hemorrhage was traced to a pea-sized aneurysm of the anterior communicating artery, which had ruptured. The heart weighed 350 Gm. The aorta showed only a few hyaline plaques. The coronary arteries were thin-walled. The kidneys weighed 310 Gm. and were soft; the capsules were somewhat adherent, and the surfaces were pale and granular.

The suprarenal glands weighed together 15 Gm., and were embedded in much fat. Attached to the left suprarenal gland by a short fibrous pedicle was an encapsulated, soft, egg-shaped mass, 4 by 2 by 2 cm. On sectioning, the color varied from reddish gray to dark purple-red, the darker shades predominating in the central portions.

Microscopic Description of the Tumor (Fig. 1).—The tumor was surrounded by a capsule of fibrillar connective tissue from which anastomosing septums extended into the tumor, subdividing it into small, variously shaped alveoli. The septums were thin and contained capillary blood vessels. In the center of the tumor were large blood-filled spaces, between which there were only a few cellular elements. There were also recent extravasations of blood and many mononuclear cells filled with iron pigment. The alveoli were composed of large polygonal cells, which lay close together, except in the places where they were separated by extravasations of blood. The cells possessed abundant, finely granular cytoplasm, which gave a very faint chrome reaction, except for a few cells which stained a deep greenish brown. The nuclei of these cells were peripherally placed and varied in size, shape and chromatin content. Many of the cells contained several nuclei. The smaller nuclei were round or oval with a fine net of small chromatin granules, a distinct nuclear membrane, and one or two distinct nucleoli. The larger the nucleus the coarser was the chromatin structure. Some of the larger nuclei were very irregular in shape. Mitotic figures could not be found.

In Giemsa-stained sections, the cytoplasm appeared light purple-pink. Scattered in the cytoplasm were often bluish-green granules about the size of a coccus. These granules in some cells were diffusely scattered; in others, they formed small clumps or clusters about the nuclei. A few of the cells were packed full with these granules.

In addition to the small granules there were larger ones which usually occurred singly, and which in the hematoxylin-stained section assumed a color similar to that of the chromatin granules. In the aniline blue-stained section the large granules appeared deep blue and were well differentiated from the purple-gray cytoplasm. The smaller granules were light grayish brown in these sections. In the iron-stained preparations, the fine granules assumed a bluish-green coloration, but the majority of them did not give the iron reaction.

The capsule which covered the surface of the tumor was in many places interrupted by cells which extended beyond the tumor into the surrounding fat tissue. Here were found cells which were somewhat different from those of the main tumor. They were oval or elongated; the nucleus was more vesicular, with a pronounced nucleolus, and many of the cells resembled sympathetic ganglion cells,

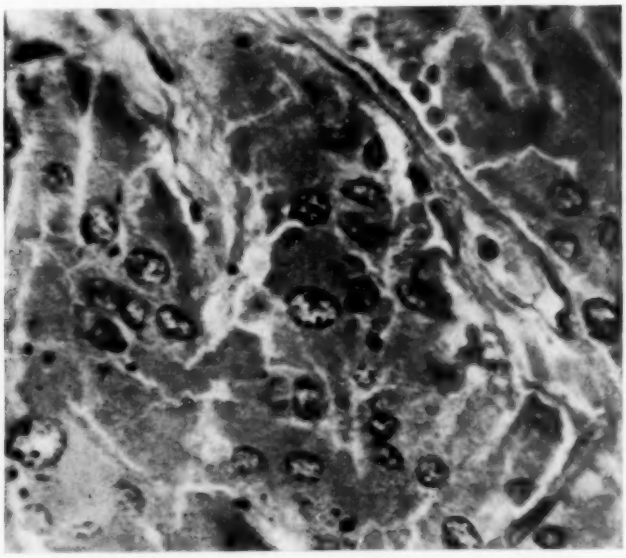

Fig. 1.—Pheochromocytoma of suprarenal gland; × 450.

although they were not surrounded by a distinct capsule. Many of the cells extended into branched processes which fused with the finely fibrillar intercellular substance. The fibrils, which were often arranged in bundles, stained purple-pink with Mallory's aniline blue method, and could be easily distinguished from the bright sky blue connective tissue. These cellular accumulations were occasionally arranged about an artery and a vein flanked by bundles of nonmyelinated nerve fibrils.

Anatomic Diagnosis.—Ruptured aneurysm of the anterior communicating cerebral artery; extensive intrameningeal and extrameningeal hemorrhage at the base of the brain; sclerosis of the basilar cerebral arteries; severe parenchymatous degeneration of the myocardium; arteriosclerosis and arteriolosclerosis of the kidneys; pheochromocytoma in the region of the left suprarenal gland; nodose goiter; obesity.

This tumor was adjacent to but outside the suprarenal gland to which it was attached and from which it received its blood supply.

There is no doubt that such tumors can arise at any point in the chromaffin system. It is my explanation that this tumor started in or just beside the suprarenal medulla, and that its growth and encapsulation gradually produced the apparent separation from the medulla.

The histologic characteristics of pheochromocytoma can be briefly summarized. There is an encapsulated tumor, made up of large cells having an abundant, finely granular cytoplasm, and a round or oval, trabecular nucleus with a single nucleolus. Multinucleated giant cells are common. The cells are grouped into alveoli by fibrous septums bearing capillaries. Hemorrhage is frequent. Occasionally smaller round, oval or spindle-shaped cells, often closely resembling lymphocytes, are seen lying in small groups. Interlacing neurofibrils are usually present. The characteristic staining quality, the chromaffin reaction, is the brown coloration of certain of the tumor cells after fixation in a solution of chrome salts or chromic acid. This reaction is normally present throughout the entire chromaffin system. The brown color is taken up by droplets within the cytoplasm and even in the nucleus of the cells and also in the stroma. Further than that there may be a brownish homogeneous coloration, irrespective of cell boundaries, which extends even into the lumens of the veins. This picture has been assumed, but not proved, to be due to the direct secretion of suprarenalin into the vein, the brown color being suprarenalin or a prosuprarenalin. Certainly the staining reaction is not a quantitative one.

That these tumors contain suprarenalin has been proved in six cases (Orth,[14] Wiesel and Neusser,[15] Wegelin,[16] Hedinger,[17] Raben [7] and Schultz [18]). These authors used Erdmann's frog's eye method or some of the chemical tests. The frog's eye method has fallen into disrepute because there are other substances in the blood plasma that may give false positive reactions. The accuracy of certain of these chemical tests seems doubtful. Among them Vulpian's ferric chloride test has been widely used, as have the reactions of suprarenalin to diluted tincture of iodine, to mercuric chloride (Comessatti) and to calcium and ammonium ferricyanide (Cevidalli). Jores' solution turns green on the addition of minute amounts of suprarenalin.

Suprarenalin Content of the Pheochromocytoma Reported.—About 1 Gm. of fresh tumor tissue was ground up with sand and 10 cc. of tenth-normal hydrochloric acid for about five minutes. The mixture was filtered and sufficient physio-

14. Orth, K.: Preuss Akad. Wissensch. **2**:34, 1914.

15. Wiesel, Josef, and Neusser, E.: Die Erkrankungen der Nebennieren, ed. 2, Vienna, M. Perles, 1910, p. 97; cited by Raben.[7]

16. Wegelin: Verhandl. d. deutsch. path. Gesellsch. **15**:255, 1912.

17. Hedinger, E.: Frankfurt. Ztschr. f. Path. **7**:112, 1914.

18. Schultz, W. H.: Am. J. Physiol. **90**:508, 1929.

logic solution of sodium chloride used to wash the filter paper to bring the volume of the filtrate to 15 cc. This extract was treated in part with permutit (an artificial saline aluminum silicate), the substance used in removing suprarenalin in the making of an extract of the cortex of the suprarenal glands for the treatment of Addison's disease (Hartmann,[19] Swingle and Pfiffner [20]). Then the pure extract and then the part treated by permutit were injected intravenously into a dog while the blood pressure was being recorded graphically. The results are shown in figure 2. The definite pressor action is strikingly present. I feel that this is a much more certain proof of the secretory power of the tumor than the chromaffin reaction. The method is not difficult and can be as easily applied as the more intricate chemical tests. The extraction of the tumor and the preparation of the kymograph tracings were done by Dr. George Milles of the Department of Pathology of the University of Illinois, who kindly granted permission for the publication of the results.

The significance of this finding is at once apparent. Evidently the chromaffin tissue subserves its secretory function independent of any close association with the suprarenal cortex. This answers in one regard Landau's [21] basic question, i. e., *"Gibt es nun auch gar keinen Zusammenhang zwischen der Funktion der Rinde und derjenigen der Marksubstanz oder besteht hier ein synergistische oder antagonistische Korrelation?"* (Is there no functional relation between the cortex and the medulla, or is there a synergistic or antagonistic correlation?) Hence the more recent trend toward distinguishing between the two parts of the suprarenal gland seems justified, and support is given to the generally accepted physiologic concept of this gland as an organ composed of two functional units.

SIGNIFICANCE OF SUDDEN DEATH ASSOCIATED WITH CHROMAFFIN TUMORS

Numerous instances of sudden death of patients with chromaffin tumors are recorded. Some are attributed to acute hypersuprarenalism (Paul [3]), because of the resemblance to death after an overdose of suprarenalin. Others are ascribed to acute paroxysms of hypertension. Sudden death is not uncommon in the various diseases of the suprarenal gland. I have seen such abrupt terminations in three cases proved post mortem to be Addison's disease. One was a case of tuberculosis of both suprarenal glands; another was a case of cytotoxic destruction of the cortex, and the third was a case of extensive hemorrhage into the suprarenal cortex on each side. Cortical tumors occasionally give rise to sudden death (Ogston [22]). Most striking is the death of a patient from whom a tumor of the suprarenal gland has been removed.

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19. Hartmann, F. A.; MacArthur, C. G., and Hartmann, W. E.: Proc. Soc. Exper. Biol. & Med. **25**:69, 1927.
20. Swingle, W. W., and Pfiffner, J. J.: Science **72**:75, 1930.
21. Landau, M.: Die Nebennierenrinde, Jena, Gustav Fischer, 1915.
22. Ogston, A., cited by Glynn: Oesterr, Jahrb. f. Paed. **1**:180, 1872.

The patient may pass the initial shock and seem out of danger, only to die unexpectedly from thirty-six to forty-eight hours later. Certain patients have been proved to have hypoplasia of the remaining suprarenal gland.

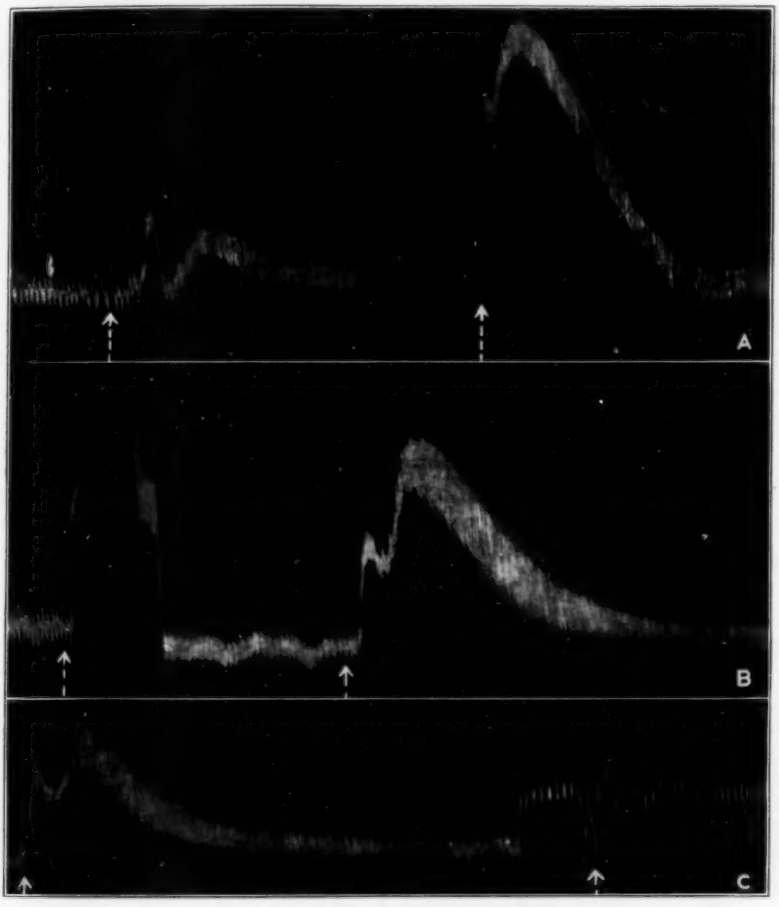

Fig. 2.—A graphic record of the effect of intravenous injection of suprarenalin, of extract of pheochromocytoma and of the same extract treated with permutit on blood pressure of a dog. For *A*, 0.1 cc. of a 1 : 10,000 solution of suprarenalin was injected at the first arrow and 0.4 cc. of the same solution at the second. For *B*, 1 cc. of tumor extract was injected at the first arrow and 0.1 cc. at the second. For *C*, 0.1 cc. of tumor extract which had been treated with permutit was injected at the first arrow and 0.1 cc. of 0.25 per cent hydrochloric acid at the second (dog coming out of anesthesia obtained with pentobarbital sodium).

HYPERTENSION

Much discussion has appeared regarding the possibility that hypertension is caused by tumor or hyperplasia of the suprarenal gland.

There is an extensive literature dating back to Josue,[23] Vaquez,[24] Krehl [25] and others. After their work the proof of a pressor substance found in the suprarenal medulla, suprarenalin, gave an impetus to that idea. The hypotension of Addison's disease contributed to it. Later it was found that repeated injections of suprarenalin into laboratory animals caused sclerosis and calcification of certain arteries, notably the aorta in rabbits. This further confused the concept by entangling arteriosclerosis with the little known rôle of the suprarenal gland in the production of hypertension, and the suprarenal action in cholesterol metabolism. Quite recently Oppenheimer and Fishberg,[26] Goldzieher [27] and Paul [3] concluded that certain cases of extreme hypertension may be due to suprarenal tumors. Goldzieher,[27] Aubertin and Clunet [28] and others found hyperplasia of the medulla of the suprarenal gland in most of the cases of hypertension which they studied, and also in cases of hypertension due to nephritis. These authors even went so far as to imply that the suprarenal gland plays a prominent part in causing arteriolar contraction of the kidney and generalized vascular changes. Paul gave the schema shown in figure 3, illustrating his ideas regarding the production of hypertension.

To support these ideas Paul cited certain cases of nephritis without hypertension. The final proof of the argument awaits the demonstration of a constant quantitative increase of some pressor substance in the blood stream derived from the suprarenal glands. Several attempts have been made to demonstrate this, but none of them has been satisfactory. The reactions for the quantitative determination of suprarenalin in the blood serum are at best rough and inaccurate. Such determinations for the suprarenalin content of the gland itself, even by Ewin's or Zangfrognini's method, are well open to question.

Oppenheimer and Fishberg pointed out ten cases with necropsy records of cortical tumors with hypertension or eccentric hypertrophy of the heart in the absence of an anatomic basis. They also cited four similar cases in which there were pheochromocytomas.

PAROXYSMAL HYPERTENSION

There are three interesting cases of paroxysmal hypertension recorded in the literature as associated with pheochromocytomas. These reports are from Labbé et al.,[12] Vaquez et al.,[10] and Shipley.[11] (The

23. Josue, O.: Bull. et mém. Soc. méd. d. hôp. de Paris **3**:48, 1908.

24. Vaquez, H., and Aubertin, C.: Compt. rend. Soc. de biol. **62**:967, 1907.

25. Krehl: Deutsche med. Wchnschr. **31**:1872, 1905.

26. Oppenheimer, B. S., and Fishberg, A. M.: Arch. Int. Med. **34**:631, 1924.

27. Goldzieher, M. A.: Endocrinology **16**:20, 1932; Virchows Arch. f. path. Anat. **280**:749, 1931.

28. Aubertin, C., and Clunet, J.: Compt. rend. Soc. de biol. **63**:595, 1907.

case of Oberling and Jung [29] is not included, because the patient died at the termination of a toxic pregnancy, the necropsy showing a "nephrite epitheliale.") In all of these patients, whose average age was 30 years, the blood pressure would suddenly rise from a normal level to 200 systolic and 130 diastolic for short periods of time. Much higher readings were made on occasion. In Vaquez's case a permanent hypertension developed from which the patient died. At autopsy there was no evidence of renal lesions. Shipley's patient with a similar case was relieved by surgical removal of a pheochromocytoma of the suprarenal gland. The explanation is advanced by these authors that sudden fluctuations in blood pressure might be due to the action of suprarenalin produced by the tumors. This is an extension of the concept of the emergency physiologic use of the suprarenal medulla according to Cannon. However, in the remaining cases of the tumor the patients were apparently not so affected, for in none of them was there any counterpart of the acute symptoms that these few patients had. In con-

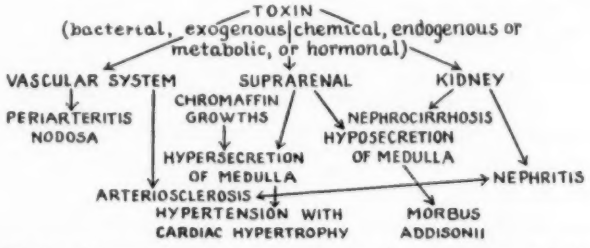

Fig. 3.—Paul's schema illustrating his conception of the production of hypertension.

trast there is the case of Mayo,[30] as well as that of Porter and Porter,[31] in which tumors near or incorporated with the suprarenal gland were found associated with paroxysmal hypertension. Neither tumor was a pheochromocytoma. The former was derived from a nerve; the latter, from the suprarenal cortex. The relief of the patients after the operative removal of the tumors established the hypertension as a sequel of the tumors. These two cases suggest that critical rises in the blood pressure may be due to mechanical pressure on the abdominal sympathetic plexus or on the splanchnic nerves. Opposed to that idea is the rarity of such a symptom complex in cases of suprarenal tumor. Further in opposition is the record of a similar blood pressure variation in a patient suffering with carcinoma of the esophagus invading the

29. Oberling, C., and Jung, G.: Bull. et mém. Soc. méd. d. hôp. de Paris **51**: 366, 1927.

30. Mayo, C. H.: J. A. M. A. **89**:1047, 1927.

31. Porter, M. F., and Porter, M. F., Jr.: Surg, Gynec. & Obst. **50**:160, 1930.

larynx without any apparent abnormality of either suprarenal gland (Harvier and Bariety [32]).

Arteriosclerosis is not to be considered as part and parcel of the hypertension syndrome. It is true that hypertension appears with arteriosclerosis of the kidneys, but in many cases at necropsy a clinical hypertension even of extreme degree known to be present for a number of years is found to be unassociated with material arteriosclerotic changes. The material in this laboratory bears out that fact almost daily. Conversely, arteriosclerosis even of a marked extent is found very commonly with no evidence of hypertension.

The idea seems generally accepted, even by Volhard,[33] that the pheochrome tumor produces hypertension as a result of chronic suprarenalin poisoning. That such is not always true is proved by this case in which a known pheochromocytoma producing an abundance of suprarenalin was not associated with any evidence of hypertension.

CONCLUSION

Pheochromocytoma is among the rarest of tumors. It is usually benign, having a characteristic microscopic picture and staining reactions.

Pheochromocytoma produces a pressor substance like, or identical with, suprarenalin, even if independent of contact with the cortex.

No connection can be validated between the suprarenal gland, its hyperplasias or tumors and the general type of essential hypertension, arteriosclerosis or nephritis.

Paroxysmal hypertension cannot as yet be explained as a phenomenon produced by transient hypersuprarenalinemia such as might arise in a patient bearing pheochromocytoma.[34]

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32. Harvier, P., and Bariety, M.: Bull. et mém. Soc. méd. d. hôp. de Paris **49**:176, 1925.

33. Volhard, F.: Nieren und ableitende, in Mohr, L., and Staehelin, R.: Handbuch der inneren Medicin, ed. 2, Berlin, Julius Springer, 1928, vol. 6, pt. 1, p. 413.

34. NOTE: Since this paper went to press an additional case of pheochromocytoma of the suprarenal medulla has been recorded by A. A. Eisenberg and H. Wallerstein (Arch. Path. **14**:818, 1932).

ABERRANT GROWTHS IN REGENERATING CLAWS OF THE HERMIT CRAB UNDER THE INFLUENCE OF SULPHYDRYL

THEIR RELATION TO NEOPLASIA

STANLEY P. REIMANN, M.D.
PHILADELPHIA

SUMMARY OF RESULTS OF PREVIOUS STUDIES OF EFFECT OF SULPHYDRYL AND SULPHOXIDE ON GROWTH

It will be remembered that in the skins of mice and rats the rate of cell division was accelerated by sulphydryl.[1] This was followed by an increase in the degree of differentiation and organization of constituent cells and tissues.[2] These findings have been confirmed by David.[3] Certain conclusions were drawn in relation to neoplasia.[4]

As another source of material for continuation of these studies the regenerating claw of the hermit crab was chosen. The Hammetts have made an extended investigation of this material.[5] But an important addition has been made. As part of the original concept, an equilibrium was postulated: If sulphydryl is the normal chemical stimulus to mitosis, the partially oxidized derivatives of this group should inhibit the process.[6]

Studies of the effects of both sulphydryl and a partially oxidized derivative, sulphoxide, on the claws of the hermit crab [5] can be summed up as follows:

1. Per unit of time, sulphydryl causes a greater than normal increase in size, and sulphoxide prevents a normal increase in size of the regenerating claws.

From the Lankenau Hospital Research Institute.

This work was carried on with the assistance of a fund provided by Mr. John T. Windrim of Philadelphia.

1. Reimann, S. P.: Protoplasma **10**:82, 1930.
2. Hammett, F. S.: Protoplasma **13**:331, 1931.
3. David, L. T.: Storrs Agricultural Experiment Station: Personal communications based on experiments with benzyl mercaptan for genetic studies: ". . . my work as having demonstrated an increased differentiation of the epidermis of adult mice following treatment with benzyl mercaptan."
4. Reimann, S. P.: Am. J. Cancer **15**:2149, 1931.
5. Hammett, F. S., and Hammett, D. W.: Protoplasma **16**:253, 1932.
6. Hammett, F. S.: Proc. Am. Phil. Soc. **64**:217, 1930; Protoplasma **11**:382, 1930.

2. These differences in total size are due to a larger and smaller number, respectively, of cells in the claws. This in turn is due to respectively increased and decreased rates of mitosis.

3. All cells are affected, but those in which the natural level of mitotic activity is highest are influenced to the greatest extent by the chemical groups.

4. Coincident with the increased number of cells, there are increased differentiation and organization of the cells and tissues. Coincident with the decreased rate of cell proliferation there are decreased differentiation and organization of the cells and tissues.

5. The three phenomena, proliferation, differentiation and organization, are so correlated that the degree and rate of differentiation per unit of time is dependent on the rate of proliferation. The degree and rate of organization of the tissue is, in turn, dependent on the degree and rate of differentiation of the cells.

6. Since sulphydryl accelerates specifically the rate of proliferation, it secondarily increases the rate and degree of differentiation and organization. Since sulphoxide diminishes specifically the rate of mitosis, it secondarily diminishes the degree and rate of differentiation and organization.

7. Differentiation and organization are the natural inhibitors of cell division.

GENERAL CONSIDERATIONS WITH RESPECT TO GROWTH

Fundamental to any increase in cell number in multicellular organisms is the sequence: proliferation, differentiation, organization. Though mutually dependent, these are, nevertheless, separate and distinct processes and must be treated as such.[7a] Also, a distinction must constantly be made between differentiation of cells and of differentiation of tissue. The term "organization" is always used of groups of cells, i.e., tissues. A break in organization, e. g., tears, injuries, irritations, stresses, strains, etc., causes proliferation. The converse, that organization inhibits proliferation, is also true. This is embodied in Ribbert's ideas of tissue tension.

From studies of mouse skin it was concluded that fully differentiated epithelial cells cannot undergo division. Wolbach's evidence is to the same effect in his studies of the replacement of one type of epithelium with another in vitamin A deficiency.[8] Therefore differentiation also inhibits cell division. But there are both degrees and kinds

7. Needham, J.: Chemical Embryology, New York, The Macmillan Company, 1931, (*a*) vol. 1, p. 541; (*b*) vol. 3, p. 1624; (*c*) vol. 1, pp. 499, 544, 596.

8. Wolbach, S. B., and Howe, P. R.: Epithelial Repair in Recovery from Vitamin A Deficiency, Experimental Study, J. Exper. Med. **57**:511, 1933.

of differentiation as well as of organization. It is the degree and not the kind of differentiation of a cell which is the important factor in inhibiting its division. The kind of differentiation is determined by the interaction of one or another or all of the cell's internal potencies with all the external influences which are called environment. For example, the epithelial cells in the basal layer of the mucosa of the trachea replace the upper layer cells as these are lost by wear and tear, etc. Ordinarily their differentiation is to cylindric ciliated cells, but in Wolbach and Howe's experiments in vitamin A deficiency (an environmental factor) certain groups of basal cells differentiated (and organized) into squamous epithelium. That is to say, the basal cells contain potencies of several kinds; which one is expressed depends on circumstances. That these potencies are internal is evident from the fact that they are expressions of their inheritance, i. e., of the lineage to which the cells belong. For further discussion of potency and examples, see reviews and texts of experimental embryology.[9] The degree to which differentiation proceeds before the ability to divide is abolished is unknown at present; certainly division is not inhibited until after the determination of which internal potency is to be expressed.

Organization is a more effective inhibitor of proliferation than differentiation; for, even though completely differentiated cells cannot divide, the process that stops the division of more undifferentiated cells is organization. So many and no more cells are required to be organized into a part, organ or tissue for replacement of wear and tear, repair of injury, etc.

If the part or organ is to remain normal, any excess of cells must be organized into a larger, but otherwise identical structure; i. e., hypertrophy and hyperplasia result.

If organization is dictated to a proper degree by none of the normal potencies of the cells or by other potencies changed in kind, proliferation continues and a tumor results.

In spite of much previous enthusiasm about a characteristic appearance of the "cancer cell," the great majority of pathologists still diagnose neoplasia and malignant growth, not on the basis of a cellular constitution, but on architecture. This means that, although differentiation of the cells is employed sometimes in naming a neoplasm, the diagnosis of the tumor, benign or malignant (even the name), usually depends on the character of the organization; that is to say, when a pathologist diagnoses malignancy, he does so on the basis of altered organization and

9. Such as Morgan, T. H.: Experimental Embryology, New York, Columbia University Press, 1927. Durken, B.: Experimental Analysis of Development, translated by H. G. and A. M. Newth, New York, W. W. Norton & Company, Inc., 1932.

says, "Here is a mass of cells which will continue to proliferate." It is tacitly insisted that cells proliferate because of altered organization. But see the excellent review and critique by Hellwig.[10]

Now an obstacle to progress in studies of growth is the lack of exact methods for measuring degrees of differentiation and organization, a fact sadly lamented by many, including Needham [7b] and Wolbach and Howe.[8] The work of Murray [11] yielded information on the quantitative relations of growth (cell division?), differentiation, "chemical growth," "chemical differentiation" and respiratory intensities. The rough estimations by pathologists of the degrees of differentiation and organization in tumors are correlated with the rates of growth of the tumors.[10]

On theoretical grounds, significant results should come from comparative measurements of material subjected to different concentrations of natural growth-stimulating and growth-inhibiting chemical substances, provided these chemical substances are definitely analyzable and controllable entities. Such substances are now provided by sulphydryl and sulphoxide as contrasted with the previously used extraneous, non-analyzable, unnatural agents tar, x-rays, etc.

In the aforementioned study of serial sections of the regenerating claws of the hermit crab, it was possible for the Hammetts to make rough but valid comparable determinations of degrees of differentiation and organization; and some abnormal growths encountered therein furnished even better material. The results are given in the summary.

In normal growth, the rates of proliferation, differentiation and organization bear an ever changing but progressive relation to each other. Immediately after cell division is concluded, differentiation is at a minimum, but rapidly increases. During differentiation of a given group of cells, the processes of organization are at a minimum, but they soon begin, overtake and exceed in intensity the processes of differentiation. That independent or consecutive or simultaneous spurts in the rates of these processes occur at various times and under various normal circumstances is well known,[9] e. g., in the organizer phenomena.

Now in the three sets of regenerating claws studied by the Hammetts, i. e., the sulphydryl, sulphoxide and control sets, it was the time factor, i. e., the rate of proliferation, which was specifically changed. There it was found that in the sulphydryl-treated claws the curves of proliferation, differentiation and organization rose to higher levels and approached each other. In the claws in which cell division was inhibited specifically by sulphoxide, the curves of proliferation, differentiation

10. Hellwig, C. A.: Arch. Path. **14**:517, 1932

11. Murray, H. A., and collaborators: J. Gen. Physiol. **9**:1, 39, 405, 603, 621, 781 and 789, 1925-1926; **10**:337, 1926.

and organization were flatter and moved away from each other. Other data on the separation of growth (cell division?), differentiation and organization come from the experiments of Broca, Dareste, Hoadley, etc. (see review by Tur [12]) in which eggs were subjected to different conditions before and during incubation, and in which various details of transplantation were modified.

A quantitative change in any one of the three processes disturbs the other two. As stated, this is shown, e. g., by the fact that a break in tissue organization causes immediate response in an effort at repair, i. e., cell division, and Hammett found that scratching a growing root tip with a needle was followed instantly by a freeing of the sulphydryl group.[13]

The speculations in a previous paper from the angle of the sulphydryl hypothesis become more valid.[4] Using as example the alternate growths of epithelial and connective tissues of the breast, I stated that perhaps the alternate influx and reflux of chemical groups concerned in differentiation and organization of the alternately growing epithelial and connective tissue tilted the level of the sulphur equilibrium now to the reduced, now to the partially oxidized, level, and thus mitosis was started and stopped. It was further stated as a fact observed in the rodent skins that increasing the rate of mitosis increases differentiation and organization. The crab claws treated with partially oxidized sulphydryl have now shown that the reverse also occurs, i. e., inhibiting mitosis results in inhibiting rates and degrees of differentiation and organization. Thus the speculation was justified as a guide to further experiments in the chemical field. These, it is obvious, are to be directed toward a study of the reactions of reduction and oxidation in naturally occurring sulphur groups.[13a]

If, as Warburg's experiments indicate, the carcinoma cell exhibits a peculiar glycolysis, the change in oxidation cannot help but be reflected to the sulphydryl group. Besides, this group being chemically more stable in acid solutions, the excess intracellular lactic acid would in itself hinder oxidation and keep the sulphur equilibrium to the reduced side. Since experiments have shown that proliferating cells other than carcinoma units, e. g., yeast cells, also produce excess lactic acid, another speculation is in order from the point of view of the sulphydryl hypothesis. Growth, structural differentiation and respiration can be separated. Perhaps the "chemical differentiation" of respiration shifts

12. Tur, J.: Compt. rend. Soc. de biol. **100**:650, 1929. Hoadley, L.: Arch. f. entwcklngsmechn. d. Organ. **116**:278, 1929.

13. Hammett, F. S.: Protoplasma **5**:547, 1929.

13a. Toennies, G., and Lavine, T.: J. Biol. Chem. **90**:203, 1931. Toennies, G., and Bennett, M. A.: To be published. Shinohara, K.: J. Biol. Chem. **96**:285, 1932.

reduced sulphur to partially oxidized sulphur and this is one of the reasons that differentiation and organization stop cell division. Data other than Warburg's are appearing,[14] but these also allow speculation with reference to the sulphydryl hypothesis.

ABERRANT GROWTHS IN THE CLAWS OF THE HERMIT CRAB

Just as distortions of growth were occasionally encountered in the skins of rats and mice treated with sulphydryl (Hammett,[2] Reimann [4]), so deviations from normal growth were encountered in a number of regenerating claws of the hermit crab. Since even casual collection, but with abnormalities in mind, of material from the beach at ebb-tide brings to light numerous specimens with peculiarities of growth, this finding was not surprising. It is the dynamic relationship of proliferation, differentiation and organization in the growths that is crucial. This is brought out in the experimental material in a way impossible in human specimens. Need attention be called to the controversies that cannot be solved by study of patients?

As part of their study of the regenerating claw of the hermit crab under the influence of the reduced sulphur-partially oxidized sulphur equilibrium, the Hammetts encountered and analyzed from the biologic point of view 44 aberrations of growth among 269 regenerating claws.[15] They have kindly given this material to me for study and analysis from the neoplastic point of view. The same procedure is followed as with the skins of rats and mice; viz., pertinent facts and conclusions are rehearsed and abstracted from their studies, additions are made in respect to neoplasia, and certain of the photomicrographs are used to illustrate the structures discussed. For complete details, the reader is directed to their papers. An important addition is, as stated, the inclusion of sulphoxide-treated specimens.

EXPERIMENTAL PROCEDURES

The right chelae were nipped off from the animals and then divided into sulphydryl, sulphoxide and control groups. They were kept in finger bowls containing sea water with the respective chemicals added. Methods of feeding, changes of water, methods of assuring oxygen supply, etc., are of the utmost importance. Detailed descriptions are given in the publications cited. Attention is again directed to the chemical pitfalls of handling these extremely labile sulphur compounds and to the necessity of using the purest samples, which are not consistently obtainable commercially.[16] The regenerating chelae were removed at intervals of from several days to two weeks and measurements of growth made. Then they were fixed in Susa's solution followed by many changes of 10 per cent formal-

14. Keilin, D.: Proc. Roy. Soc., London, s.B, **104**:206, 1929.

15. Hammett, F. S., and Hammett, D. W.: Protoplasma **17**:321, 1932.

16. Dr. Gerrit Toennies has checked constantly and often purified the reagents.

dehyde, embedded by the double method of de Renyi, cut serially at 7 microns, stained with Heidenhain's iron hematoxylin stain, and mounted in neutral balsam. Over 25,000 serial sections thus prepared have been studied.

DESCRIPTION OF THE ABERRANT GROWTHS, WITH COMMENT

The 44 disturbances of growth were divided into groups 1, 2 and 3 on the basis of the underlying mechanisms:

1. The stump of the amputated claw quickly becomes enveloped in chitin. A defect in this layer allows unorganized growth to protrude. Five of this kind were encountered.

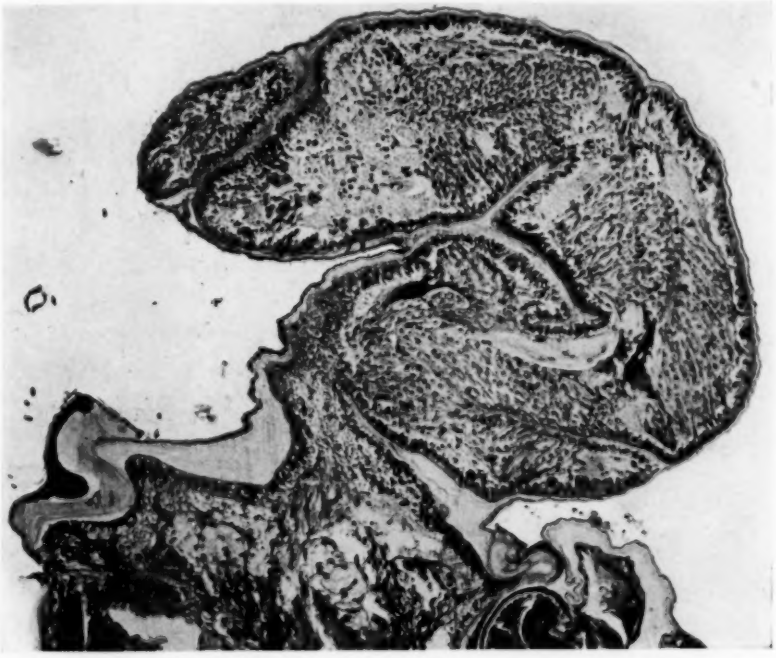

Fig. 1.—Sulphydryl-treated normal regenerating claw; × 95. Note (1) the active proliferation, differentiation and organization and their relations; (2) the thinner chitin on the dorsal surface, and (3) segments being determined.

2. In the regenerating claw, the chitin layer is thinner over the dorsal surface and is especially thin on the dorsal aspects at the junctions between segments of the growing claw and between new claw and old stump (fig. 1). The activities of living lead to tears in these structurally weaker areas, and cellular overgrowths protrude. Thirty of this kind were encountered (figs. 2, 3 and 4.)

3. At the tip of the distal segment, aberrant growths sometimes arise. There were nine of these (figs. 5 and 6 *A*).

The growths arising in groups 1 and 2 are secondary to the accidental break in the confining envelop of chitin. Loss of mechanical restraint, among other factors, allows cells to exhibit proliferative powers which are not expressed under normal circumstances; i. e., it

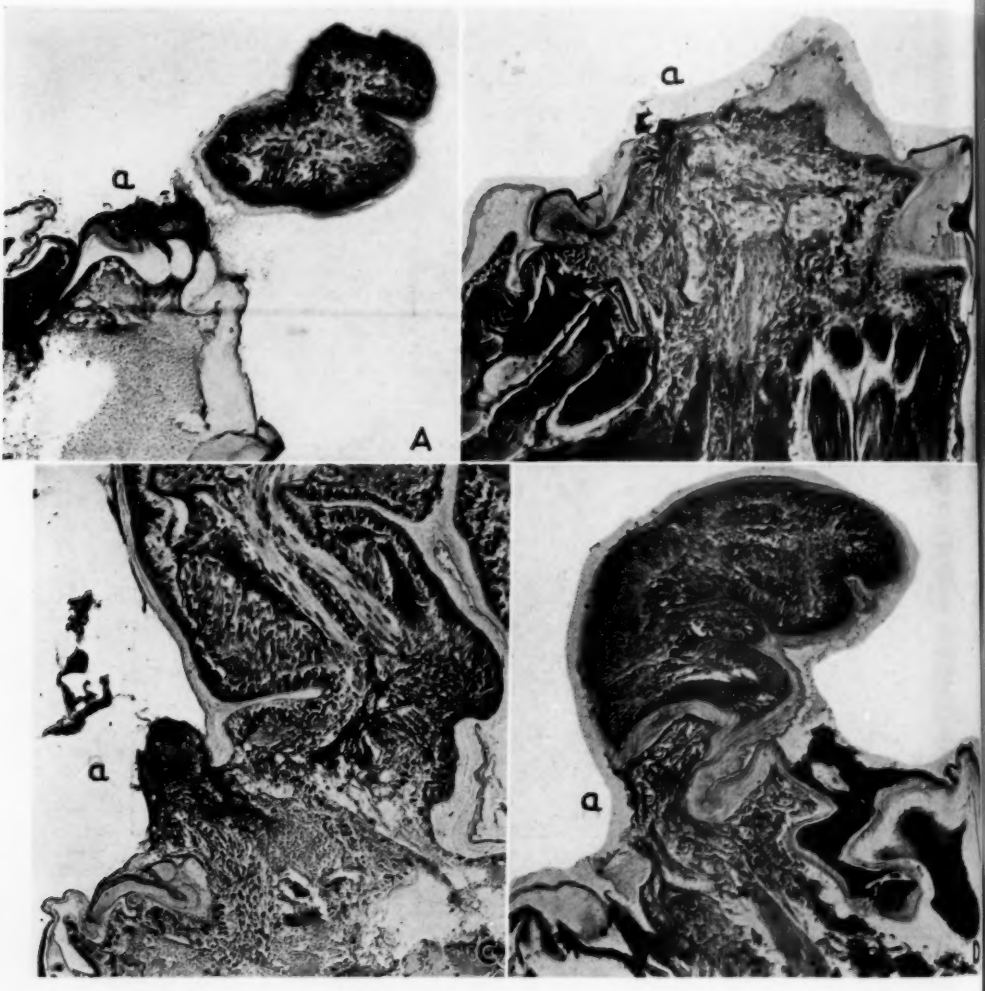

Fig. 2.—*A*, control claw; × 95. A small unorganized growth protrudes through a defect in the chitinous envelop of the sheath at *a*. *B*, sulphoxide-treated claw; × 95. No growth emerges through the defect in the chitin at *a*. *C*, control; × 95. A small growth emerges through the tear at the junction at *a*. *D*, control; × 95. No growth emerges through the tear at *a*.

breaks organization; hence these growths emerge through tears in the chitin. That these tears are not artefacts produced in the preparation of the sections is demonstrable, since in all the artefacts the exposed

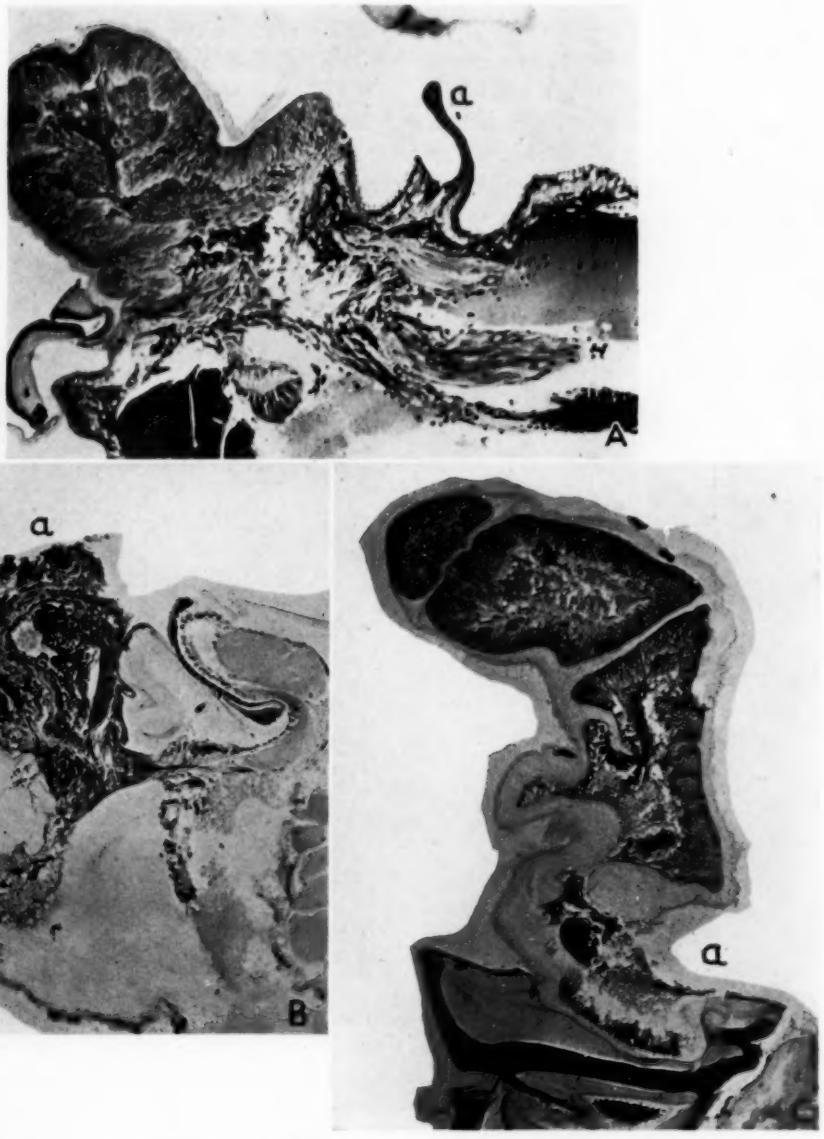

Fig. 3.—*A*, control; × 95. An artefact is shown. No growth is seen. A strip of chitin is pulled off at *a*. *B*, sulphydryl-treated claw, with large growth at the tear at *a*; × 95. Note that both differentiation and organization are active. *C*, sulphoxide-treated claw; × 95. No growth emerges through the tear at *a*.

tissue is organized and covered with the epithelium which was under the chitin when it was torn off, and the partially adherent strip of chitin can be seen. Also, the criterion constantly used in histologic diagnosis is present; i. e., a tissue reaction occurs which could not have taken place if the tear had been made during embedding, etc.

The growths are attempts at repair. But why is more tissue produced than is needed, when a few more epithelial cells and some new chitin are all that is necessary? Chitin is a product of epithelial cells, and these cells must reach a certain degree of "functional organization" before chitin is produced.

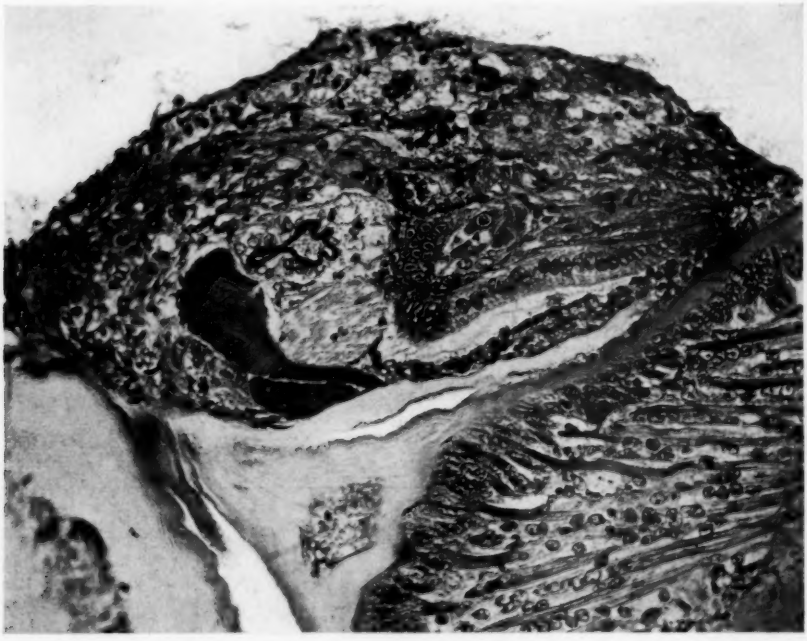

Fig. 4.—Sulphydryl-treated claw, with growth through a tear in the chitin; × 250. Differentiation of the cells is advancing, and organization is beginning.

Proliferation, differentiation and organization are very active in the claw as a whole. They are mutually dependent, although their relative rates differ. If the break in the chitin occurs at a time when proliferation is at its height, the differentiation and especially the organization processes are insufficiently developed to prevent excess cells from being produced; the path of least resistance is through the break, and the proliferating cells stream through. Therefore, the rate at which proliferation is taking place when the break occurs determines the number of cells and thus the size of the growth. Since different times elapsed between the occurrence of the breaks and removal for fixation of the

new regenerates, various stages of differentiation and organization should be found in the growths, provided the potencies of differentiation and organization are normal and are being asserted. Also, if sulphydryl is the specific stimulant of division, in addition to the growths being larger, they should be present more frequently in the sulphydryl-treated

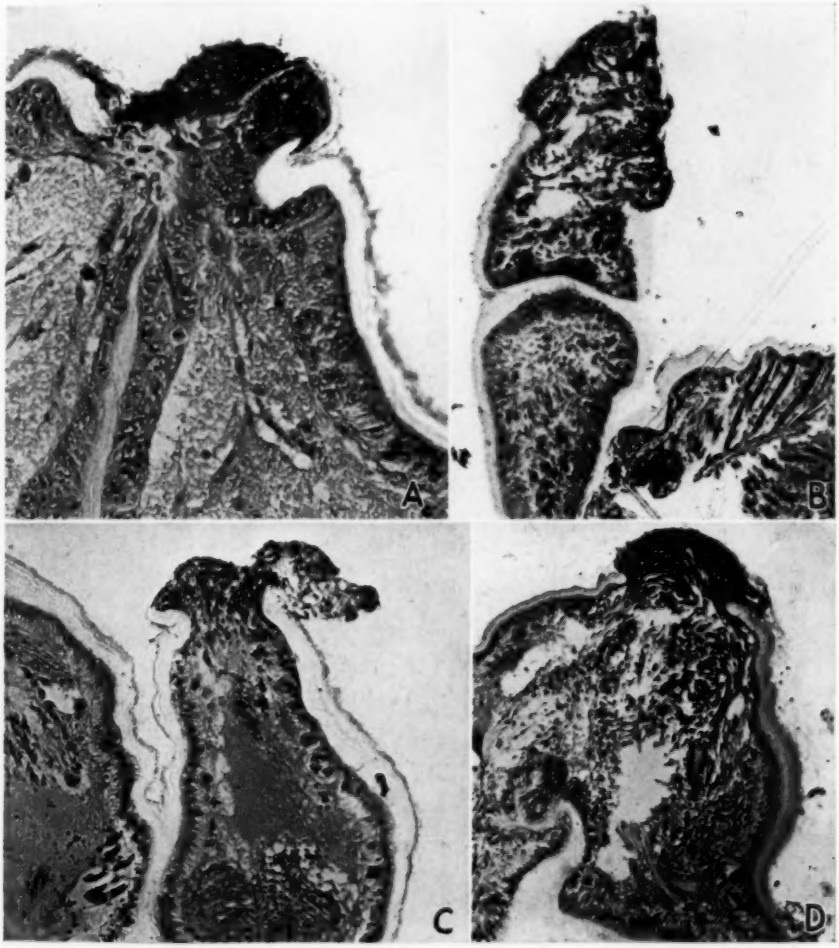

Fig. 5.—*A*, sulphydryl-treated claw showing growth from the tip of the propus, with a heaping up of epithelial cells and a delay in chitin formation. *B*, *C* and *D*, sulphydryl-treated claws each showing a growth from the tip of the propus, in which differentiation is proceeding to the formation of epithelial cells, muscle cells and connective tissue cells. Organization is delayed, but is manifestly beginning.

claws than in the controls or in sulphoxide-treated claws, since sulphydryl not only increases the intensity of cell division, but also

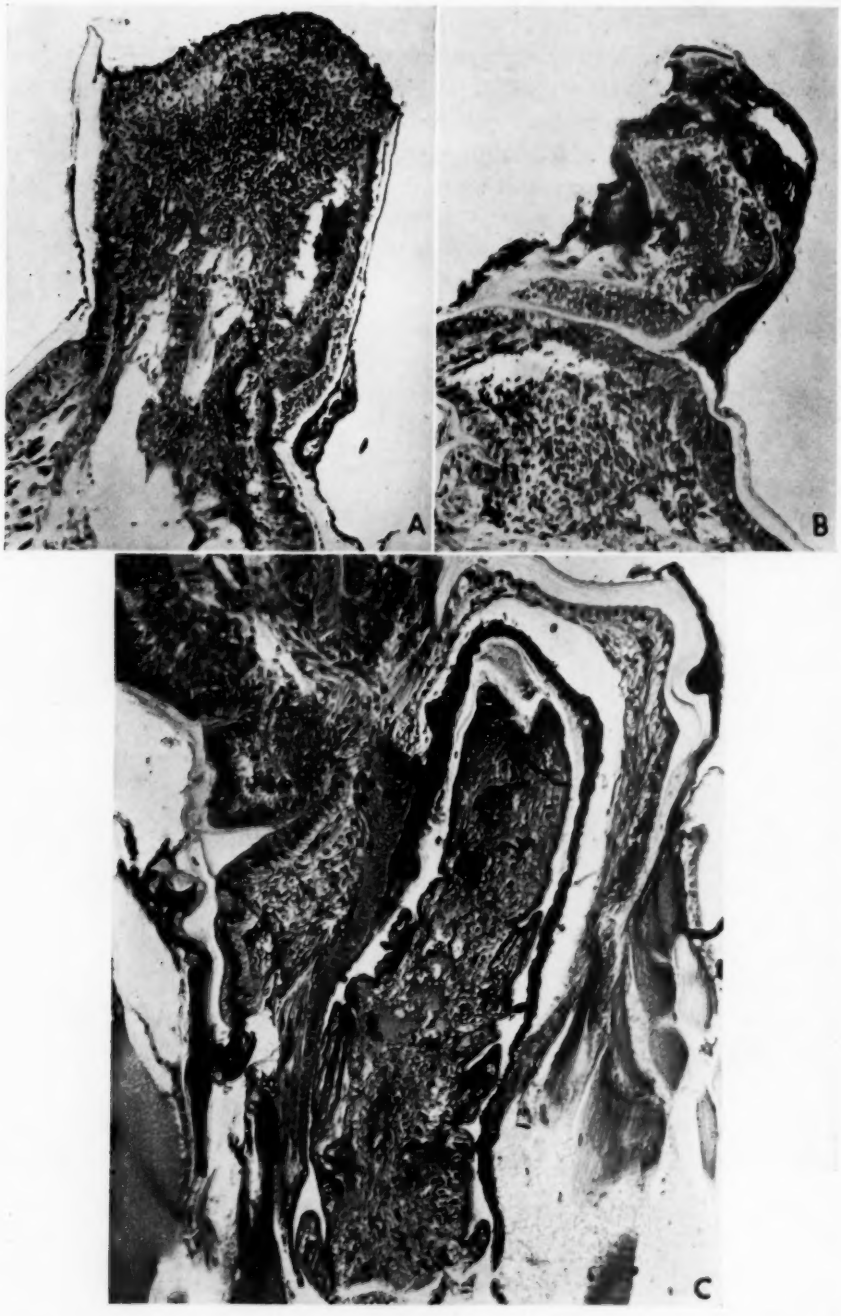

Fig. 6.—*A*, sulphydryl-treated claw. A scattering of the "growth center" of the propus leads to much proliferation, but delayed differentiation and organization. *B*, sulphydryl-treated claw showing hyperchitinosis of the propus from an excess of epithelial cells and possibly their higher differentiation. *C*, sulphydryl-treated claw showing a second regenerate attempting to differentiate and organize within the stump of the old claw.

influences to the greatest degree those cells which are naturally dividing at the highest rate. Conversely, in the sulphoxide-treated specimens, cell division being inhibited, fewer and smaller growths should be found than in the controls. This is precisely the case, for in the sulphydryl-treated claws, 100 per cent of those injured showed growths, while, of the controls, 75 per cent reacted, and, of the sulphoxide-treated specimens, 43 per cent reacted.

Another biologic fact must be considered; viz., that after proliferation, differentiation and organization have advanced to a certain degree, the species-specific factors enter to mold the growth into the proper structural and functional form for a hermit crab's claw. Again it is to be expected that variations of time between production of the break in the chitin and fixation of the specimen will determine these details.

As stated, all of this is contingent on the internal potencies of differentiation and organization, which must be normal. The photographs show that both of these processes are present in the growths and in various stages, and are proceeding structurally normally; that is, epithelial cells are being oriented to the surface, chitin is being produced, and connective tissue and muscle are being differentiated and oriented.

The reason that more cells were produced than were needed for simple repair is, therefore, that the removal of a mechanical restraint and the intrusion of a new set of ratios needed for repair into the normal ratios already active for regeneration shifted the normal time relations between proliferation, differentiation and organization. Since the older growths show reassertions of differentiation and organization, and in the normal direction, it follows that the growths result from changes in degree and not from changes in kind of the normal processes of development.

Perhaps overrepair as in keloids, dense adhesions, etc., is due to shifts in these time relations started by an increased rate of cell division due to increased sensitivity to division or to increased concentration of the division-stimulating chemical group.

The difference of these growths arising as a result of the external accidents of living from malignant growths is obvious. In neoplastic disease, differentiation and especially organization are changed, not only in degree, but also in kind. For these growths to be malignant, the internal potencies of cell differentiation and especially of organization must be foreign to those imparted by a normal inheritance.

The third type of growth encountered, viz., that from the tip of the distal segment, the propus, differs entirely from the other two. It occurred only in the sulphydryl-treated specimens, never in the controls or in the sulphoxide-treated claws. Nine such growths were

encountered in the 138 sulphydryl-treated specimens. There was no tear or other mechanical causative factor.

Now the newly developing propus is the site of a "growth center" in which mitosis is active in a nest of closely packed epithelial cells. The directional stimulus (growth gradient) of this "center" is distal to the old stump. The cells produced in this "center" of the normal propus undergo structural differentiation and organization into which there are intruded functional differentiation and species-specific organization to produce a perfect new propus for the claw of the hermit crab. But in order to produce a segment perfect for the species in the average time, it is necessary not only that these processes be normal in quality, but also that they be timed normally. Shifts in their absolute and relative rates will produce claws different from the normal in *degree* but not in *kind*. For, when normal and "shifted" specimens are compared at the same intervals of time, the retarded claws appear like normal claws of an earlier age, and the stimulated claws appear like normal claws of a later age.

More experiments are needed to discover whether the superiority and inferiority, respectively, of the sulphydryl-treated and sulphoxide-treated claws would be maintained until and beyond completion of regeneration or whether their development would be slowed or accelerated, respectively, to make the end-results of the same size as normally regenerated claws. At present, therefore, there remains this difference between the effects of sulphydryl on rodent skins and crab claws: In the rodent skins, the degree of complexity of differentiation and organization is greater than ever occurs normally in the adult animals; in the crab claws, the evidence thus far indicates, it is greater per unit of time. The distinction between degree per unit of time and degree per species per age is important in ontogeny and phylogeny, and it is equally important in neoplasia, for the crab claws confirm the first two conclusions derived from the rodent skins,[4] which are: *"The experimental forcing of cell division with the natural chemical stimulus, -sulfhydryl-, results not in diminished but in increased differentiation and organization of the cells so forced,"* and *"Increased rate of cell division alone never leads to malignancy."*

Now the crucial question to be answered in determining whether the 9 propus growths are neoplastic or not is whether differentiation and particularly organization are merely shifted in their relations or whether they are changed in kind also. Or, put in another way, do the proliferating cells show at least beginning normal differentiation and organization and response to the species-specific organization impulses which they contain within themselves by virtue of their inheritance as hermit crab cells?

The serial sections show unequivocally that differentiation and organization are being asserted, not only in all degrees, but also in the normal direction. Here and there, epithelial cells are being oriented in normal positions; spicules of chitin are being produced; connective tissue and muscle are being differentiated normally. The series leaves no doubt that if these growths were given time, they would smooth out and become organized.

For the growths to be considered as neoplastic it is necessary to find evidence of changes in kind of potencies for differentiation and organization. If present, these changes must have occurred within the cells.

Again an answer is given to the question: What is to be expected when excess cells are produced by proliferation? If the chemical and physical make-up is normal, the result will be a larger normal part, but if this make-up is not normal, the result will be growths of various degrees of aberrancy which never recover perfect differentiation and organization, and which therefore continue proliferating.

Another conclusion in the previous paper derived from entirely different material is thus sustained: *Malignant cells differ from normal cells in the quality of their chemical composition as far as the factors of differentiation and organization are concerned.*

Since these propus growths are not tumors, the following interpretation is offered for their presence: Normally, as stated, the segment contains a "growth center" producing the requisite number of epithelial cells at the proper rate to produce a new part. Surrounding the growth center are other cells which differentiate into muscle and connective tissues, etc., at a period of time just after the production of the epithelial cells. The production of chitin is a function of the epithelial cells and is accomplished only after they have reached a certain degree of differentiation and organization. Organizers compel the epithelial cells reaching a sufficient degree of functional differentiation to produce chitin and deposit it in the normal peripheral position. This, in turn, as an organizer of the second degree, acts as a barrier and organizer in the completion of the claw. If, however, cells are produced too rapidly in the growth center, those reaching the chitin-producing stage may be numerically insufficient to produce enough chitin to prevent overflow of the excess; i. e., there is not a sufficient number of adequately differentiated cells. Hence the excess cells stream out from the tip, this being the normal direction in which growth takes place (growth gradients), and aberrant growth is the result.

Since these growths are found only in the sulphydryl-treated claws, it follows that *sulphydryl is capable of stimulating proliferation to the production of aberrant, unorganized growths.*

To correct and inhibit further misunderstanding, due mostly to failure to separate clearly the biologically distinct functions of proliferation, differentiation and organization, the following point is emphasized: If sulphydryl is specifically a normal chemical stimulant to mitosis, it will not, indeed it theoretically should not, by itself lead to the production of neoplasms. It should (and does) accelerate the rate of cell division; what becomes of the cells so stimulated depends on the quality of their internal potencies or on their lack of them, plus their environment.

Two other specimens, both sulphydryl-treated, deserve special mention. In one, the propus is composed exclusively of epithelial cells, and an excessive amount of chitin is deposited on the surface. The biologic interpretation of this is that the "growth center" was stimulated by sulphydryl to produce a larger number of epithelial cells. Their differentiation was also accelerated as usual, but the rate of differentiation relative to that of proliferation was so accelerated that "functional differentiation" occurred very early; the resultant deposition of chitin in the normal situation checked proliferation of connective tissue and muscle. The excess of chitin was produced by the larger number of cells and may have been due also to increased degrees of differentiation (fig. 6 *B*).

Chitin is the homologue of keratin. Of the numerous examples of keratosis in man, two only need be mentioned for comparison, leukoplakia and the keratinizing squamous cell carcinoma.

Both rodent skins and crab claws [15] demonstrate that the inherent potency for differentiation of epithelial cells is greater than is usually expressed under normal circumstances. The upper limit of differentiation of squamous epithelial cells seems to be their production of and transformation into keratin. In leukoplakia, both proliferation and differentiation are increased in degree, for there are more cells and more keratin; so also in keratinizing squamous cell carcinoma. But, in the crab claw and in leukoplakia, the organizing factors are such that the orientation of the cells remains normal, and proliferation slows down. In keratinizing squamous cell carcinoma, however, organization is changed in kind and thus proliferation continues.

In the other specimen, two new claws are being regenerated, one of normal shape and in normal position, the other within the stump of the old claw (fig. 6 *C*). Obviously two anlagen for regeneration were stimulated to active growth. Expressed in terms of potency, two cells or two groups of cells containing within them the potencies for producing all the structures of a new claw were stimulated to proliferation. The one cell or group of cells which produced the semblance of a claw within the old stump expressed these internal potencies as well as the

environment permitted. Experienced pathologists will have no difficulty in citing many identical occurrences in human material. The literature of experimental embryology teems with examples found by workers in this subject, especially in their experiments with transplantation, explantation and interplantation. Wolbach and Howe's results in vitamin A deficiency are again quoted[8] to emphasize the effect of environment on the form of expression of the internal potencies of cells. But, lest the environment be thought too powerful, it is emphasized that cells are extremely resistant to changes in environment and have enormous powers of adaptation. This was cleverly shown recently by Parker with tissue cultures in which he transplanted fibroblasts from different situations and ages into different environments and noted their return to type when they were again placed in the original mediums.[17]

This inclusion in the crab claw shows no invasion, excellent differentiation and good organization in the proper direction. It is not a neoplasm, but a developmental anomaly. To have been a neoplasm it must have had abnormal potencies within its cells. Changes in environment in the presence of proliferating cells of normal internal composition may produce distortions of growth, teratologic structures, etc.,[7c] but the term neoplasia should not be applied.

To summarize the considerations thus far:

1. When normal cells are stimulated to increased rates of proliferation, increased rates and degrees of differentiation and organization follow but always as an expression of one or other of the potencies inherited by the cells as part of their normal internal complement.

2. The environment helps to determine which of the potencies is to be expressed and its degree.

3. The three fundamental properties of cells, viz., proliferation, differentiation and organization, are separate but mutually dependent biologic functions.

4. Differentiation and organization are the natural inhibitors of proliferation.

5. Failure in qualitative differentiation and organization is due to altered internal composition of the cells.

As a basis for correlating these conclusions with the observations on benign tumors, the common glandular and connective tissue tumor of the breast, the periductal fibroma, may be chosen. Many others could be used, e. g., the adenoma of the thyroid gland so ably discussed by Graham,[18] who decided that he would call it a tumor and not mere

17. Parker, R. C.: Science **76**:446, 1932.

18. Graham, A.: Am. J. Surg. **7**:163, 1929.

hyperplasia—after discussing the shortcomings of the differential criteria in these cases.

For the histories and details of a group of cases of periductal fibroma, an article by Ingleby may be read.[19] Further roentgenologic and histologic studies along the lines previously reported by Reimann and Seabold[20] bear out Ingleby's conclusion that errors in normal growth are concerned in cases of this tumor.

Briefly, in the periductal fibroma differentiation of both epithelium and connective tissue is little disturbed; even functional and species differentiation are almost but not quite perfectly attained in many cases. The tumor responds to menstrual impulses in a manner similar to that of the normal breast, and in many instances it has been known even to lactate. The difference between the tumor and the normal breast is in its architecture, i. e., in its organization, just as in adenoma of the thyroid gland.[18] As a rule the tumor increases in size slowly and yet perceptibly; in some cases, it becomes stationary after reaching a certain mass.

These facts indicate that a failure of the proper time relations of the alternating organizations of connective tissue and epithelium of the breast *cause* the tumor. There has been a qualitative change in the "reaction basis" of the cells affecting at least the powers of organization. The potencies which are expressed are qualitatively different from those expressed by the normal breast. Since this change is slight, the organization which occurs retards but does not prevent proliferation. Hence the result is a tumor which grows slowly.

Malignant tumors exhibit varying rates of proliferation and various degrees of altered differentiation. Tumors, whether benign or malignant, if composed of cells of high degrees of differentiation, are known to grow more slowly. It is because of these high degrees of differentiation that this is so. In some cancers, the majority of the cells are differentiated to the point of actual function; e. g., in adenocarcinoma of the large intestine, they produce mucus. Completely differentiated cells do not divide; there are relatively fewer division-capable cells in such tumors. What organizing power is present is expended on the arrangement of the tumor cells themselves into glands, etc., and none is left for interaction with other tissues except as is necessary for nutrition. They are capable of no response to species-specific and other influences. Thus the degree of internal change of composition of the cells is apparently greater in malignant tumors than in benign, for their organization is poorer. The degree of malignancy,

19. Ingleby, H.: Arch. Path. **14**:21, 1932.

20. Reimann, S. P., and Seabold: Am. J. Cancer **17**:34, 1933.

i. e., the rate of proliferation, therefore, depends on the degree of alteration of the potencies of differentiation and organization within the cell.

The fact that the less a tumor is organized, and the less its cells are differentiated, the higher is the rate of proliferation is basic evidence that the laws of development are in essence the same for neoplasms as for normal growth. The difference lies in their expression. This fact, not usually recognized, is the main basis of tumor grading, a subject in which, it is gratifying to note, the clinical application is being recognized as general and not specific to the individual case.[10]

Malignant growth is usually the proliferation of one kind of cells, epithelial or connective tissue, etc., as the case may be. Some of the aberrant growths of the propus of the crab's claw contain only epithelial cells; others contain, in addition, connective tissue and muscle. But the serial sections show that it is the epithelium which opens the way, and that the connective tissue follows or is dragged after it.

Regarded broadly, epithelium has so evolved that the combination of highly specialized function and necessity of replacement is enjoyed by this tissue more than by any other. The constant necessity of replacement increases the chances of error, i. e., tumor formation, in epithelium, and this is an old story in the literature on tumor. It is a biologic truism that the development of highly specialized function on the part of one individual requires that assistants do some of the other work. Epithelium, wherever situated, requires connective tissue at least to bring it nutrition, but connective tissue being low in specialization can exist without epithelium. Hence there is always connective tissue in carcinomas, but no epithelium in sarcomas. The connective tissue, or stroma, to use the word so heavily endowed with the controversies of years, is not neoplastic. It does not continue to grow after the epithelial cells which it accompanied in the carcinoma are destroyed by irradiation. It continues to organize, as it should, into a scar. The potencies of its cells are normal, but cannot be expressed to full degree because of the environment; i. e., it accompanies epithelial cells with altered potencies. In this sense, the stroma of a carcinoma is a developmental anomaly.

My aversion to considering the connective tissue in and around carcinomas as evidence of a specific "immune reaction" is stated elsewhere, with the reason for it. I see no reason to alter my opinion.[21]

A problem frequently presented to the practical pathologist is illustrated by the study of the growths in these crab claws. So often it is the time factor which is the crux of the situation in the practical diag-

21. Reimann, S. P.: Arch. Path. **8**:803, 1929.

nosis of tissue, for it would be easy to determine the identity of borderline lesions if series of sections taken at various intervals of time could be examined. As an example, in the edges of gastric ulcers, epithelium is often displayed in the midst of granulation tissue, and the question arises: Is this repair or carcinoma? What would have happened if the surgeon had not removed the ulcer when he did? In terms of the present observations, the problem can be presented in this way: If only the relative rates have been shifted, it is not carcinoma; if a change of the normal potencies has occurred, it is a neoplasm. Thus in the crab claws, if sections had been made at only one time (early), there would be every justification for calling the overgrowths neoplasms. Since, however, serially examined specimens show that differentiation and organization have set in and are of normal kind, the growths are in no sense neoplasms.

Therefore, since differentiation and organization are normal inhibitors of cell division, and since quantitative shifts in their normal relations merely cause changes in the time of inhibition of proliferation, it follows that continued proliferation of any group of cells is caused by qualitative changes in their internal composition.

It cannot be argued that the time factor is not sufficient in neoplastic disease, i. e., that proliferation is so rapid that differentiation and organization cannot overtake it, for it is a rare tumor, indeed, which grows as rapidly as normal tissues. Besides, recurrences of malignant growth often appear after cells not removed by operation, etc., have lain dormant for years.

Now it has been shown that the degrees of the changes in differentiation and organization of the cells of a tumor are directly correlated with the degree of change in the rate of proliferation. But since even in long-standing neoplastic growth there is no organization, it must be answered that it is internal qualitative changes within the cells which determine benignancy and malignancy of neoplasms and the degrees of these qualities, i. e., their rate of growth.

Metastasis, i. e., the independent proliferation of cells carried to a strange environment, is possible because the brakes to proliferation, viz., differentiation and organization, have been removed. These absences must be inside the cells, for metastasis occurs anywhere, including the very same environment in which other cells are proliferating, differentiating and organizing and also regressing normally.

The "cell autonomy" of the literature on tumors and of the tissue culturists who figure how many days or weeks it would take for a fragment of tissue, if it could be let loose, to proliferate to a mass of cells the size of the earth becomes the statement: If the cells do not differentiate and organize, they do not stop proliferating.

It goes without saying that the internal changes in neoplastic cells which prevent proper differentiation and organization must be transmitted from mother to daughter cells as they undergo division.

Viewed in these lights, the facts unearthed by those experimenting in the problem of potency, incomplete though those facts may be, are seen to relate to several extremely controversial points in neoplasia. To say the least, they render many views veritable William of Occam's razors. As stated, fully differentiated epithelial cells no longer divide (Hammett;[2] Reimann;[4] Wolbach and Howe[8]). Replacement is from undifferentiated multipotential cells in the basal layers. This finding that there are probably always present, and naturally, incompletely differentiated cells capable of going on to division shows that it is no longer necessary to postulate the presence of "embryonal rests" when squamous cell carcinoma arises in the gallbladder or when islands of stratified squamous epithelium are found in the urinary bladder, to mention two of many examples.

The term "metaplasia," correctly defined, is the display of a potency other than the usual one for the part or the organ.

The term "embryonal" applied to a cell means that the potency of the cell remains to be determined; applied to a tissue, the term means that many potencies of the tissue remain to be determined. Its use with respect to neoplasia is to be deprecated, for neoplastic cells and tissues do not have normal potencies as contrasted with embryonal cells and tissues.

The biologic finding that aberrant growths arise from a "growth center" in the propus finds its correlation in the observed development of neoplasms from growth centers and points of transition of one to another type of epithelium. It again demonstrates the biologic unity of growth, development and neoplasia.

Finally, to lead to the repetition of another conclusion of a previous paper,[4] the only known way of increasing the incidence of spontaneous tumors is by breeding. In deference to experimental embryology, and until such time as experimental embryologists and geneticists agree, the term "chromosome" will be changed to "reaction basis," which for the time being includes the chromosomes. The conclusions become:

By inheritance certain cells have "reaction bases" of unstable composition. The instability is one of degree requiring much and long continued irritation in some cases, and possibly none at all externally applied in others, to cause the ultimate actual internal changes which precede neoplasia.

IN RE: SULPHYDRYL HYPOTHESIS

Previous considerations in regard to neoplasia as a problem in the chemistry of cell division will be found in Hammett's paper of 1929.[22]

22. Hammett, F. S.: Arch. Path. **8**:575, 1929.

Since, in the absence of differentiation and organization, proliferation continues, the sensitivity of cells to division remains. It has been shown that the greater the natural tendency to cell division, the greater is the response to sulphydryl. Since sulphydryl is a normal stimulus to cell division, it follows that the proliferation of malignant growth is inherently related to the sulphydryl group.

Three possibilities are open: 1. As a direct effect of the derangement of the chemical groups controlling differentiation and organization, the sulphur equilibrium is thrown to the reduced side; i. e., there is more sulphydryl per cell.

2. The alteration in the chemical groups of differentiation and organization permits the cell to respond to smaller amounts of sulphydryl; i. e., there is hypersensitivity.

3. An increased amount of sulphydryl or an increased sensitivity keeps the cells from differentiation and organization; i. e., a shift in the level of the reduced sulphur-partially oxidized sulphur equilibrium determines the change in the chemical groups of differentiation and organization.

DEFINITION OF A NEOPLASM

The following generalization emerges in the form of a definition of neoplasm.

A neoplasm is a mass of cells which arise from and continue to proliferate within an organism as a result of and in direct proportion to their degree of internal qualitative differences from the other cells of the organism with respect to the potencies of differentiation and organization particularly.

SUMMARY

Aberrant growths arising in the regenerating claws of the hermit crab when the claws were exposed to sulphydryl and sulphoxide have been studied in their relation to neoplasia.

In both the aberrant growths and the normal regenerating cells, sulphydryl specifically stimulated the rate of cell division, while sulphoxide specifically retarded the rate of cell division. This was followed by increase and decrease, respectively, of the rates of differentiation and organization of the cells so stimulated and retarded.

These growths in the crab claws were not neoplasms, because there was no qualitative change in the potencies of the cells. The significance of change in degree and in kind of potencies is discussed.

The normal relations of proliferation, differentiation and organization as determined by the internal potencies of the cells were disturbed quantitatively in these growths, but not qualitatively as in neoplasms.

Sulphydryl specifically stimulated "growth centers" to the production of aberrant, unorganized growths.

A generalization is given in the form of a definition of neoplasm.

CONCLUSIONS

Experimental increase of the rate of proliferation of the cells by means of their natural chemical stimulus—sulphydryl—results in increased rates and degrees of differentiation and organization of the cells so stimulated. Experimental decrease of the rate of cell division with suboxidized sulphydryl results in decreased rates and degrees of differentiation and organization.

An increased rate of cell division alone can never lead to malignant growth.

By inheritance certain lines of cells contain potencies of various degrees of instability. In some individuals a slight, in others a greater, impetus (irritation) is required to produce the internal qualitative changes necessary for malignant growth, and in some individuals perhaps no impetus needs to be applied externally.

When the changes in the potencies of differentiation and organization are developed, they are transmitted from mother cells to daughter cells.

The normal inhibitors of proliferation are differentiation and especially organization. If the internal potencies for differentiation and organization of the cells are qualitatively different from the normal for the lineage, differentiation and organization are not accomplished and hence proliferation continues.

ETIOLOGY OF GALLSTONES

EUGENE L. WALSH, M.D.
CHICAGO

Of the many theories of gallstone formation, the stasis-infection theory of Naunyn,[1] the stasis-hypercholesteremia theory of Aschoff and Bacmeister[2] and the physicochemical theory of Lichtwitz[3] have gained the greatest popularity. Several modifications of these theories have arisen. Recently new theories of stone formation have been presented by Whitaker,[4] Halpert[5] and Sweet.[6] Andrews, Schoenheimer and Hrdina[7] have presented evidence for the most recent theory of stone formation. They maintain that bile salts may diffuse out of the gallbladder, allowing the cholesterol, which the salts hold in solution, to crystallize out and form stones.

RECORDED OBSERVATIONS ON THE SOLUTION OF HUMAN GALLSTONES PLACED IN THE GALLBLADDERS OF ANIMALS

Because of reports that human gallstones dissolve when placed in the dog's gallbladder, it was thought that a study of this phenomenon might yield some information concerning the etiology of these calculi in man.

Labes[8] was the first to observe that gallstones dissolved in the dog's gallbladder. Naunyn[1] later confirmed this. Hanseman[9] showed that mixed stones, that is, the cholesterol-pigment-calcium variety, dissolved but that those of calcium did not. Frerichs[10] and Quincke[11] have also shown this. Wilhelm,[12] Bain,[13]

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

1. Naunyn: Klinik der Cholelithiasis, Leipzig, F. C. W. Vogel, 1892; English translation by Garrod, London, The New Sydenham Society, 1896.
2. Aschoff and Bacmeister: Die Cholelithiasis, Jena, Gustav Fischer, 1909.
3. Lichtwitz: Deutsches Arch. f. klin. Med. **92**:100, 1907.
4. Whitaker: J. A. M. A. **88**:1542, 1927.
5. Halpert: Arch. Path. **6**:623, 1928.
6. Sweet: West Virginia M. J. **23**:617, 1927.
7. Andrews, Schoenheimer and Hrdina: Proc. Soc. Exper. Biol. & Med. **28**:944, 1931; Arch. Surg. **25**:796, 1932.
8. Labes, quoted by Hanseman.[9]
9. Hanseman: Virchows Arch. f. path. Anat. **212**:139, 1913.
10. Frerichs, F. T.: Klinik der Leberkrankheiten, Braunschweig, F. Vieweg u. Sohn, 1861, vol. 2, p. 487.
11. Quincke, quoted by Hanseman.[9]
12. Wilhelm: Wien. klin. Wchnschr. **31**:696, 1918.
13. Bain, quoted by Leo.[14]

Leo [14] and Harley and Barrat [15] showed that stones were dissolved by the normal dog's gallbladder. The latter observers found that the stones were not dissolved if the gallbladder was infected with bacteria of the colon group introduced at the time of operation. In the more recent literature, Harrison and Barber,[16] Walsh and Ivy [17] and Mirolli [18] have again observed that human gallstones placed in the gallbladder of the dog disappear. Aoyma [19] has found that human gallstones are dissolved in the cat's and rabbit's gallbladder as well as in the dog's.

Glaesner [20] stated that the rate of solution of the stone in the dog depended on the size and the variety of stone used. He did not find that cholesterol added to the diet significantly altered the rate of solution. He found that the addition of cysteine to the diet caused a more rapid dissolving of the stone. Neuberg and Friedman [21] showed that cysteine may be changed in the body to taurine. From his experiments Glaesner concluded that bile salts were important for the solution of the stones.

Workers in Japan recently called attention to the relation of fat-soluble vitamins to gallstone formation. Fujimaki [22] reported the formation of calculi in the kidney, urinary bladder and biliary tract in rats fed on a diet low in these vitamins. Usuki [23] reported gallstone formation in rabbits fed on a diet deficient in vitamin A. The stones contained calcium, bilirubin and cholesterol, but were only the size of millet seeds. Nakashima and Saheki [24] found that gallstones from cattle decreased in size in the dog's gallbladder, provided a diet rich in vitamin A was fed (five experiments). The same authors [25] found that the same was true for human gallstones in the dog's gallbladder.

Hedinger [26] and also Frerichs [10] spoke of the dissolving action of the human gallbladder on stones. In a collection of stones from one gallbladder examined by me, eroded edges of considerable depth were noted on two stones. This erosion was assumed to be due to solution, which would confirm the foregoing reports.

EXPERIMENTAL OBSERVATIONS ON SOLUTION OF HUMAN GALLSTONES PLACED IN THE DOG'S GALLBLADDER UNDER VARIOUS CONDITIONS

In the experiments in this work, mixed gallstones, that is, the cholesterol-pigment-calcium variety, were used. These were kept immersed in 0.9 per cent sterile salt solution and dried with a sterile towel just prior to weighing. They were then replaced in salt solution after being weighed in air. Just prior to placing a stone in a dog's gallbladder, the external surface was sterilized by a brief immersion in 95 per cent alcohol. The stone was then placed in the dog's gall-

14. Leo: Deutsche med. Wchnschr. **16**:786, 1914.
15. Harley and Barrat: J. Physiol. **29**:341, 1903.
16. Harrison and Barber: Proc. Soc. Exper. Biol. & Med. **25**:226, 1927.
17. Walsh and Ivy: Ann. Int. Med. **4**:134, 1930.
18. Mirolli: Arch. ital. di chir. **30**:314, 1931.
19. Aoyma: Beitr. z. path. Anat. u. z. allg. Path. **57**:168, 1913.
20. Glaesner: Wien. klin. Wchnschr. **31**:549, 1918.
21. Neuberg and Friedman, quoted by Rosin: Ztschr. f. physiol. Chem. **124**: 282, 1922-1923.
22. Fujimaki, quoted by Usuki.[23]
23. Usuki: Jap. J. Gastroenterol. **1**:18, 1929.
24. Nakashima and Saheki: Jap. J. Gastroenterol. **1**:134, 1929.
25. Nakashima and Saheki: Jap. J. Gastroenterol. **3**:271, 1931.
26. Hedinger: Schweiz. med. Wchnschr. **45**:1037, 1921.

bladder with aseptic technic. The dogs were fed on various diets. The standard diet consisted of yellow corn-meal, bread and bone soup. At first the rate of solution was followed by the Graham-Cole [27] technic of gallbladder visualization. When the shadow of the stone became small, the stone was removed. Later 65 days was set as an arbitrary period of observation.

In a previous communication, Walsh and Ivy [17] reported that in dogs on a standard diet the stones lost above 90 per cent of their weight in from 130 to 150 days. In 65 days, the loss was about 56.7 per cent, the maximum being 87 per cent, the minimum 24.8 per cent. When olive oil was added to the diet, the average loss was 53.6 per cent, the maximum being 86.8 per cent, the minimum 21.1 per cent for the 65 days. The addition of 2 ounces (62.2 Gm.) of coconut oil daily to the diet gave for the period an average loss of 60.5 per cent, the maximum being 100 per cent, the minimum 35 per cent. The presence of marked cholecystitis caused the loss in weight to be much less for the period. In five dogs in which the cystic duct was ligated at the time of the introduction of the stone into the gallbladder, the changes in weight varied between a plus and a minus 5 per cent

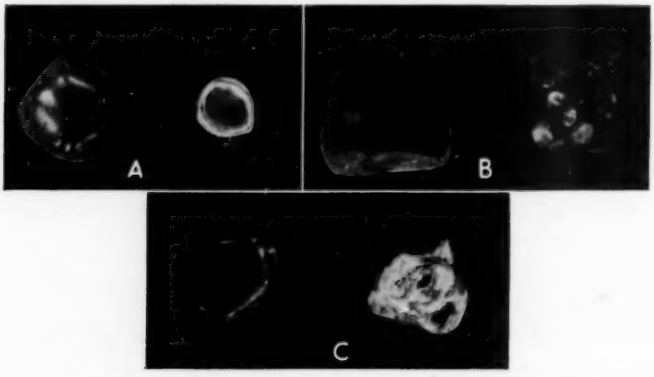

Fig. 1.—Human gallstones before and after sojourns in the gallbladders of dogs for sixty-five days: stone *A* weighed 1.292 mg. before and 0.178 mg. afterward. Stone *B* weighed 1.535 mg. before and 0.272 mg. afterward. Stone *C* weighed 1.551 mg. before and 0.883 mg. afterward.

for the 65 days. When the cystic duct was ligated without the introduction of a stone into the gallbladder, at the end of 2 months the wall of the gallbladder was in most cases thickened, and the gallbladder contained calcium-pigment-carbonate granules suspended in a clear or light-colored, very thick mucinous mass. In a series of animals with stones in the gallbladder and a mechanical stricture of the common bile duct, the average loss of weight in the stones in 65 days was 44 per cent. Stricture of the common bile duct alone in a series of dogs led in every case to the presence of from 50 to 100 mg. of calcium-pigment-carbonate sediment in the contents of the gallbladder. Cholecystitis produced by chemical means (Dakin's solution [28] or alcohol) did not significantly alter the rate of solution of stones in the dog's gallbladder. The cholecystitis so produced was either not chronic or not sufficiently diffuse to prevent concentration of the bile.

27. Graham and Cole: J. A. M. A. **84**:1563, 1925.
28. Mann: Ann. Surg. **73**:54, 1921.

That the solution of the stones begins early is shown in table 1. In these experiments the stones remained in the gallbladders less than 65 days. Some loss of weight occurred in stones that remained in gallbladders only 5 days.

Since lauric acid is one of the chief acids of coconut oil, and since in vitro lauric acid was found to be the best gallstone solvent (vide infra), a series of five dogs was used to test this acid as a solvent when added to the diet. Five grams of solid lauric acid was fed daily for 65 days. The average loss of weight of the stones was 56 per cent, the maximum being 78 per cent, the minimum 41 per cent. This rate of solution was not greater than that of the controls.

Since it is reported by Japanese workers that the addition of fat-soluble vitamins to the diet of dogs aids in the solution of stones in their gallbladders, it

TABLE 1.—*Early Solution of Human Gallstones in the Gallbladder of a Dog Less Than Sixty-Five Days (Dog on Stock Diet)*

Stones	Days	Original Weight, Mg.	Loss in Weight, Mg.	Loss, per Cent
1	5*	1.882	0.125	6.6
2	13	2.952	0.099	3.3
3	5*	1.710	0.078	4.5
4	15	1.657	0.242	14.6
5	15	1.756	0.126	6.1
6	3*	1.467	0.019	1.2
7	45	0.856	0.155	18.0

* Note that solution begins early.

TABLE 2.—*Effect of Adding 60 Cc. of Cod Liver Oil Daily to the Stock Diet on the Rate of Solution of Gallstones Placed in the Dog's Gallbladder*

Stone	Days	Original Weight, Mg.	Loss in Weight, Mg.	Loss, per Cent	Comment
1	65	0.846	0.622	73.5	
2	65	0.788	0.428	54.5	
3	65	1.790	1.340	74.8	
4	65	0.981	0.669	68.0	Gallbladder moderately inflamed; frothy bile
5	65	0.973	0.451	46.5	
6	65	1.282	1.012	79.0	Frothy bile
7	65	1.094	0.766	70.0	
Average		1.108	0.755	66.6	

was decided to repeat this work. Sixty cubic centimeters of cod liver oil was given daily for sixty-five days. The results are shown in table 2. The average loss in weight of the stones was 66.6 per cent. The maximum was 79 per cent, the minimum 46.5 per cent. This rate of solution was slightly greater than that of the controls.

Since a diet high in cholesterol may influence the biliary cholesterol,[29] a diet of one-half pound (226.7 Gm.) of calf brains, one egg, 1 ounce (28.3 Gm.) of butter and 3 ounces (85 Gm.) of bread was fed to each of three dogs daily for sixty-five days. This diet did not alter significantly the rate of solution. The average loss of weight of the stones for the period was 57 per cent.

Bile salts, especially sodium taurocholate, are known to exert a considerable cholagogic effect. This has been reported to be of significance in the dissolution of gallstones placed in the dog's gallbladder.[20] A series of nine dogs was fed

29. d'Amato: Biochem. Ztschr. **69**:216, 1915.

1.5 Gm. sodium taurocholate (an impure commercial preparation obtained from the Wilson Laboratory) daily for sixty-five days. The results are recorded in table 3. The average loss in weight in the stones was 62.5 per cent, the maximum being 99.8 per cent, the minimum 13 per cent. This rate of solution was greater than that of the controls, even with the inclusion of two stones which on removal

TABLE 3.—*Effect of Feeding 1.5 Gm. of Sodium Taurocholate Daily on a Gallstone in the Dog's Gallbladder*

Stone	Days	Original Weight, Mg.	Loss in Weight, Mg.	Loss, per Cent	Comment
1	66	1.564	1.301	83.5	Wall of gallbladder hypertrophied
2	70	1.419	1.394	99.8	
3	70	1.899	1.521	80.4	Wall of gallbladder hypertrophied
4	75	1.016	0.689	68.9	
5	60	1.175	0.153	13.0*	Bile flaky, almost colorless; stone impacted; encrusted with yellow precipitate
6	60	1.512	1.322	87.5	
7	60	1.346	0.387	28.6	Wall of gallbladder hypertrophied
8	60	1.202	1.057	87.3	
9	60	1.201	0.164	13.6*	Stone encrusted with yellow material; bile flaky; stone in cystic area; bile thin and almost colorless
Average.....		1.370	0.888	62.5	

* If these two stones are omitted, the average is 76 per cent.

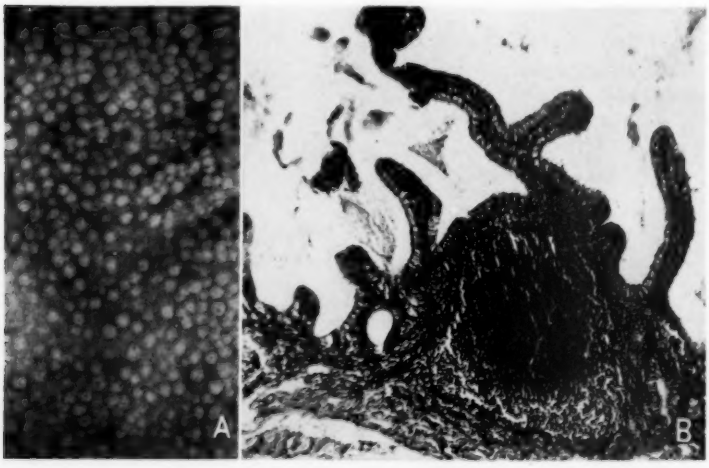

Fig. 2.—*A*, lymphoid hyperplasia of the gallbladder of a dog in which a human gallstone had been placed; the dog was fed on a stock diet plus 60 cc. of coconut oil daily. *B*, a microscopic section of mucous membrane.

were encrusted with a pigment-calcium-carbonate coat, and which were in a gallbladder filled with a flaky sediment and thin, almost colorless bile. Both of the stones appeared to be impacted in the area of the cystic duct, and from the findings in the gallbladder, they must have prevented to a considerable extent the flow of bile to and from the gallbladder. This was particularly true of stone 5, which was adherent to the mucosa. Omitting these two stones from the series, one

arrives at an average solution of 76.5 per cent, which is the most striking result obtained in the various attempts to increase the rate of solution of stones placed in the dog's gallbladder.

It is evident from these experiments that human stones placed in the dog's gallbladder slowly dissolve, that this solution begins early, and that it is prevented more or less if definite cholecystitis is present or if there is an impairment of bile flow to and from, or of the concentrating activity of, the gallbladder. Also, it is evident from these experiments that stones from the same patient, while essentially of the same composition, dissolve at different rates in different dogs. When the same stone is placed in the gallbladders of different dogs, the rate of solution varies. These results are probably due to varying degrees of cholecystitis in different dogs and to varying degrees of interference with the concentrating activity of the gallbladder, the motility of the bile tract and the formation of bile in different dogs. Because of these variables, conclusions can be drawn only in a general way from such experiments and cannot be based on small percentage changes in weight.

In an attempt to confirm Aoyma's report [19] that gallstones disappear in the rabbit's and the cat's gallbladder, "mixed" stones were placed in the gallbladders of these animals and observed for periods varying from thirty-three to seventy-two days. Three cats and two rabbits were used. The stones lost only about 0.5 per cent in weight in the cat's gallbladder. In the cat, the wall of the gallbladder hypertrophied, and the stones became impacted in the cystic duct, as Whitaker [4] had observed previously. This may account for the slight amount of solution observed. In the rabbit's gallbladder, the stones increased in weight 3.6 per cent. The wall of the gallbladder was thickened. Probably here also the filling and concentrating activity of the gallbladder was interfered with.

SUMMARY OF FOREGOING OBSERVATIONS

A summary of the results of the ten series of experiments is given in table 4. The stones in the control group showed an average loss of 56.7 per cent. Olive oil, coconut oil or lauric acid added to the diet did not appreciably alter the rate of solution. Neither did a diet high in cholesterol. Acute chemical cholecystitis did not alter the rate of solution, since diffuse chronic cholecystitis was not obtained. The daily administration of sodium taurocholate gave an average loss in weight of the stones of 62.5 per cent (possibly 76 per cent), which is somewhat greater than normal. This confirms the observation of Glaesner.[20] The daily feeding of cod liver oil gave an average loss in weight of the stones of 66.6 per cent, which is greater than the normal rate of solution and confirms the report of Nakashima and Saheki.[24] Although the increases in rate of solution of the stones when cod liver oil and sodium taurocholate were fed are not striking, it is believed that they are significant. It is believed that sodium taurocholate acts through its cholagogic effect by increasing the total amount of bile coming into contact with the stone. I have no explanation to offer in regard to how cod liver oil might act, since the effect of cod liver oil on bile composition and flow is not known.

Ligation of the cystic duct reduced the rate of solution to almost zero. A mechanical stricture of the common bile duct gave an average solution of 44 per cent, which is considerably below the normal. The bile is the factor which dissolves the gallstones. Anything preventing bile flow into and out of, or concentration by, the gallbladder, will alter the rate of solution of human gallstones placed in the dog's gallbladder.

OBSERVATIONS ON THE SOLUBILITY OF HUMAN GALLSTONES IN VITRO

In the literature, there are several reports on the solvent action of various solutions on human gallstones. Brockbank [30] showed that oil, fatty acids and sodium and potassium soaps were good solvents of cholesterol. Happel [31] found that bile salt and soap solutions had cholesterol-dissolving power. Rosin [32] found that

TABLE 4.—*Summary of Results of Placing Gallstones in Dog's Gallbladder for Sixty-Five Days*

	Number of Experiment	Average Original Weight, Mg.	Average Change in Weight, Mg.	Average Change, per Cent
Control on stock diet	9	1.513	0.920	56.7
60 cc. olive oil daily	10	2.101	1.083	53.6
80 cc. coconut oil daily	9	1.650	0.949	60.5
5 Gm. lauric acid daily	5	1.512	0.852	56.3
0.5 lb. calf brains, 1 egg, 1 oz. butter, 3 oz. bread daily	3	2.834	1.615	57.2
50 cc. cod liver oil daily	7	1.142	0.797	66.6
1.5 Gm. sodium taurocholate daily	9	1.475	1.226	62.5*
Ligation of cystic duct	5	1.043	0.009	— 0.8
Cholecystoduodenostomy with cystic duct tied	1	0.887	0.024	— 2.7
Stricture of common bile duct	4	1.380	0.500	44.0
Stones in peritoneal cavity	2	1.481	+0.003	+ 0.2
Stones in cat's gallbladder	2	0.441	—0.002	— 0.5
Stones in rabbit's gallbladder	2	0.132	+0.005	+ 3.6

* If the two stones that were impacted are omitted, the average is 76 per cent.

mixed human gallstones were dissolved by bile salt solutions, and that sodium desoxycholate had from three to four times the solvent action of other bile salts. Herter [33] reported that the solvent action of bile for cholesterol depends on the biliary soaps and fat.

That bile itself has a solvent action in vitro is shown in several reports. Aoyma [19] found that human stones lost weight in cholesterol-poor bovine bile, whereas they increased in weight in human bile. Loewy and Glaser [34] reported that bile from dog and human fistulas when allowed to run over human gallstones, caused a slight decrease in weight if a preparation "chologen" had been given previous to the collection of the bile. More recently Bauman and Spanner [35] reported that the saturation values of various biles for cholesterol vary. Dog bile

30. Brockbank: On Gallstones or Cholelithiasis, Philadelphia, P. Blakiston's Son & Co., 1896, p. 67.

31. Happel, quoted by Naunyn.[1]

32. Rosin: Ztschr. f. physiol. Chem. **124**:282, 1922-1923.

33. Herter: Tr. Cong. Am. Physicians & Surgeons **6**:158, 1903.

34. Loewy and Glaser: Cor.-Bl. f. schweiz. Aerzte **38**:377, 1908.

35. Bauman and Spanner: Proc. Soc. Exper. Biol. & Med. **28**:730, 1931.

has the highest value, ox bile has the next highest value, and human bile the least value.

The experiments on animals show that there is some principle in, or some fraction of, the bile in the dog's gallbladder which is responsible for the solution of human gallstones of the mixed cholesterol variety when the stones are placed in it. In the attempt to find the fraction responsible, various solutions of known biliary constituents were used, the results being recorded in tables 5 and 6. In these experiments, human gallstones of the mixed variety were used. As in the experiments on animals, the stones were kept immersed in salt solution and dried immediately before weighing. They were then tied in small pieces of gauze to prevent mechanical erosion and placed in 50 cc. Erlenmeyer flasks. The solutions to be tested were introduced in 10 cc. quantities into the flasks and changed daily unless otherwise noted. If the solution to be tested was not water-soluble, it was used as an emulsion or a suspension. The flasks were placed in a shaking machine, which consisted of a windshield-wiper pump attached by a supporting rod to a ring-stand. The flasks were placed in buret clamps on the machine, and the speed was so regulated as to give about sixty alternate half revolutions per minute. The apparatus was placed in an oven held at 37 C. At the conclusion of the experiment, the stones were removed; they were washed with water, their surfaces dried and their weights recorded. In earlier experiments, a fifteen day test period was used (table 5); later a five day period was used (table 6).

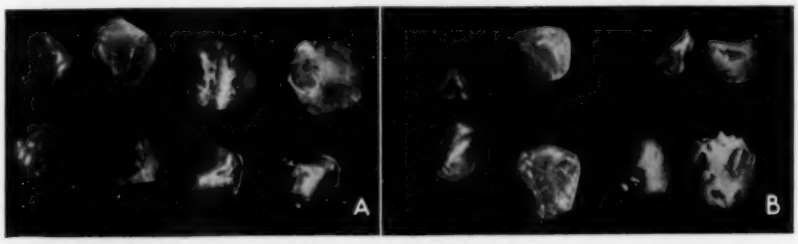

Fig. 3.—Human gallstones before and after being shaken for fifteen days in a p_H 8 buffer solution with 1 per cent oleic acid: *A* weighed 1.441 mg. before and 0.968 mg. afterward. *B* weighed 1.050 mg. before and 0.725 mg. afterward.

A partial report of some of this work has appeared earlier.[17] Much of the work, however, has been extended and is reported here. In water, in 0.9 per cent salt solution and in p_H 8 phosphate buffer solution there is practically no solution for the period, showing that mechanical agitation alone cannot account for the losses found. In bile from a human fistula, bile from a human gallbladder, bile from a patient with hydrops, bile from a hog's gallbladder, bile from a dog's hepatic fistula, bile from a dog's gallbladder diluted ten times with water, 1 per cent sodium cholate in water, 1 per cent sodium glycocholate in water, 1 per cent sodium taurocholate in water or 1 per cent sodium dehydrocholate in water, there is practically no change in weight. Since bile from a dog's gallbladder which is diluted ten times with water shows little dissolving properties, it cannot be the cholesterol concentration of the solvent per se that causes the solution to take place. It is to be noted that bile from a dog's gallbladder gives about 26 per cent solution of the stone in 15 days, and that bile from a dog's fistula concentrated ten times dissolves about 54 per cent. Concentration of bile is unquestionably a factor in the solvent action. The fact that the bile of the dog's gallbladder was probably not concen-

TABLE 5.—*Effect of Shaking Gallstones in Vitro with Various Solutions at 37 C. for Fifteen Days* *

Solution Used	Number of Experiment	Average Original Weight, Mg.	Average Loss in Weight, Mg.	Average Gain or Loss, per Cent	Maximum Change		Minimum Change	
					Weight	Per Cent	Weight	Per Cent
Distilled water	6	0.714	+0.017	+ 2.3	+0.119	+10.3	—0.003	— 0.7
0.9% sodium chloride	8	1.173	+0.008	+ 0.7	+0.082	+ 5.9	—0.003	— 0.5
p_H 8 buffer solution	1	0.866	+0.001	+ 0.1	.....	.....	.....	.....
Bile from human fistula	4	2.348	+0.002	+ 0.1	+0.003	+ 0.7	.....	.....
Bile from patient with hydrops	2	0.866	—0.003	— 0.3	+0.007	+ 0.2	—0.002	— 0.1
Bile from human gallbladder	8	1.108	+0.002	+ 0.1	+0.009	+ 0.7	—0.005	— 0.3
Bile from beef gallbladder	8	0.378	—0.068	—18.2	—0.117	—35.4	—0.038	— 9.7
Bile from sheep's gallbladder	6	0.424	—0.073	—17.2	—0.100	—23.3	—0.050	—11.4
Bile from hog's gallbladder	6	0.743	—0.002	— 0.2	—0.005	— 1.2	—0.000	— 0.0
Bile from dog's gallbladder	18	1.110	—0.290	—26.0	—0.638	—62.3	+0.006	+ 1.9
Bile from dog's fistula	8	0.897	—0.014	— 1.5	—0.030	— 4.3	—0.002	— 0.4
Bile from dog's fistula concentrated 10 times by evaporation	5	0.539	—0.292	—54.5	—0.436	—76.8	—0.170	—23.1
10% aqueous solution of bile from dog's gallbladder	7	0.909	+0.026	+ 2.8	+0.096	+ 8.5	—0.003	— 0.3
Boiled bile from dog's gallbladder	4	0.725	—0.286	—37.0	—0.630	—67.5	—0.046	—14.1
1% sodium cholate in water	2	0.568	+0.000	+ 0.0	.....	.....	.....	.....
1% sodium taurocholate in water	7	1.169	+0.020	+ 1.6	+0.128	+ 9.3	+0.005	— 0.3
1% sodium glycocholate in water	5	1.414	+0.023	+ 1.6	+0.107	+ 8.0	—0.000	— 0.0
1% sodium dehydrocholate in water	1	0.996	—0.001	— 0.1	.....	.....	.....	.....
1% sodium cholate + 1% sodium taurocholate in water	2	0.493	—0.033	— 6.7	.....	.....	.....	.....
1% butyric acid in p_H 8 buffer	5	1.140	+0.008	+ 7.7	+0.150	+14.0	+0.006	+ 0.5
1% butyric acid in p_H 6 buffer	5	1.120	+0.104	+ 9.3	+0.310	+29.8	+0.044	+ 4.8
1% oleic acid in p_H 8 buffer	19	1.207	—0.202	—16.7	—0.263 —0.483	—67.0 —33.1	—0.090 —0.062	— 4.1 —13.6
1% oleic acid in p_H 6 buffer	19	0.987	—0.155	—15.7	—0.429 +0.045	—33.3 + 5.3	—0.036	— 2.9
1% lauric acid in p_H 8 buffer	17	0.752	—0.548	—73.0	—0.337 —0.852	—100.0 —83.2	—0.322 —0.550	—66.4 —62.3
1% myristic acid in p_H 8 buffer	11	0.942	—0.241	—25.6	—0.363 —0.331	—34.2 —36.8	—0.144	—12.1
1% lauric acid + 1% sodium cholate in p_H 8 buffer	4	0.732	—0.646	—88.4	—0.688	—100.0	—0.516	—63.7
1% lauric acid + 1% sodium taurocholate in p_H 8 buffer	6	0.776	—0.540	—69.6	—1.016 —0.300	—69.2 —83.4	—0.231	—47.1
1% lauric acid + 1% cholesterol in p_H 8 buffer	7	0.954	—0.409	—42.8	—0.829	—58.9	—0.401 —0.207	—27.8 —37.4
1% lauric acid + 1% cholesterol + 1% sodium taurocholate in p_H 8 buffer	4	0.684	—0.196	—28.8	—0.261	—32.8	—0.119	—20.8
1% lauric acid + 1% cholesterol + 1% sodium taurocholate in p_H 8 buffer	6	0.648	—0.114	—17.4	—0.219	—33.4	—0.061 —0.068	— 9.9 — 6.8

* The solutions were changed daily; 10 cc. amounts were used.

TABLE 6.—*Effect of Shaking Gallstones in Vitro with Various Sloutions at 37 C. for Five Days* *

Solution Used	Number of Experiment	Average Original Weight, Mg.	Average Loss in Weight, Mg.	Average Gain or Loss, per Cent	Maximum Change		Minimum Change	
					Weight	Per Cent	Weight	Per Cent
Bile from dog's gallbladder	4	1.011	—0.194	—19.1	—0.252 —0.174	—15.3 —26.1	—0.252 —0.158	—15.3 —16.9
Bile from dog's gallbladder, dialyzed 48 hr. against tap water and evaporated to original volume	1	0.843	—0.168	—20.0	.....	.....	.....	.....
Bile from dog's gallbladder dialyzed 100 hr. against tap water and evaporated to original volume	5	0.754	—0.026	— 3.4	—0.064	— 8.5	—0.019	— 1.8
6% sodium cholate in p_H 8 buffer	5	0.873	—0.066	— 7.6	—0.058 —0.076	—14.3 — 4.7	—0.058 —0.076	—14.3 — 4.7
6% sodium taurocholate in p_H 8 buffer	6	0.727	—0.023	— 3.2	—0.043 —0.026	— 6.0 — 7.5	—0.005	— 0.5
10% sodium taurocholate in water	6	0.865	—0.051	— 5.8	—0.043 —0.085	—16.7 — 8.1	—0.021	— 2.1
10% sodium taurocholate saponified, acidified, extracted with petroleum ether, neutralized, in water	3	0.876	—0.075	— 8.5	—0.106	—11.0	—0.057	— 6.5
10% sodium taurocholate acidified, extracted with petroleum ether, neutralized, in water	4	0.882	—0.057	— 6.4	—0.089	— 6.8	—0.043	— 5.7
1% oleic acid in p_H 8 buffer	6	0.794	—0.053	— 6.7	—0.045 —0.082	—13.1 — 9.6	—0.041	— 4.1
1% linseed oil soaps in water	3	0.823	—0.336	—40.8	—0.420	—54.6	—0.159	—23.6
1% lecithin in p_H 8 buffer	5	0.771	+0.002	+ 0.3	+0.008	+ 0.9	—0.002	— 0.3
6% sodium cholate + 1% oleic acid in p_H 8 buffer	6	0.675	—0.051	— 7.5	—0.056 —0.069	—13.8 — 5.5	—0.049	— 4.2
6% sodium taurocholate + 1% oleic acid in p_H 8 buffer	6	0.637	—0.056	— 9.5	—0.067 —0.091	—20.2 — 7.7	—0.026	— 3.3
10% sodium taurocholate + 1% oleic acid in water	4	0.714	—0.124	—17.3	—0.114 —0.182	—28.2 —19.8	—0.092	—11.9
6% sodium cholate + 1% oleic acid + 1% lecithin in p_H 8 buffer	5	1.068	—0.084	— 7.8	—0.139	—13.5	—0.039	— 3.2
6% sodium taurocholate + 1% oleic acid + 0.5% cholesterol in p_H 8 buffer	2	0.821	—0.017	— 2.0	.....	.....	.....	.....
6% sodium cholate + 1% lecithin in p_H 8 buffer	5	0.849	—0.042	— 4.9	—0.046	— 7.2	—0.030	— 3.4
6% sodium taurocholate + 1% lecithin in p_H 8 buffer	5	0.794	—0.041	— 5.2	—0.053	— 8.8	—0.031	— 3.5

* The solutions were changed daily; 10 cc. amounts were used.

trated ten times in that organ is the explanation of these differences. The same explanation will hold for the bile from a dog's gallbladder which had been boiled, wherein a 37 per cent solution occurred. There was some concentration of the bile in the boiling process, and the bile may have been more concentrated to begin with. This experiment shows that the dissolving capacity of the bile is not due to enzymatic activity.

Beef bile and bile from the sheep's gallbladder are shown to have considerable solvent capacity.

With various fatty acids as solvents, it was found that the stones increased in weight in butyric acid solutions, but lost markedly in oleic, lauric or myristic acid solutions of a 1 per cent concentration in a p_H 8 buffer solution. The rate of solution was best in the lauric acid solution, being 73 per cent for the 15 days. There is evidence of better solvent action at a more alkaline reaction. This is best explained on the basis of there being more soap in solution to hold the fatty acid in aqueous solution. The fatty acid is apparently the most active principle, since it can be demonstrated in a test tube that pure fatty acids dissolve gallstones readily, whereas neutral fats or bile salts do not have such marked solvent action. (The experiment is to place a gallstone in oleic acid and heat. The stone readily dissolves.)

The addition of bile salts to a 1 per cent lauric acid solution in a p_H 8 buffer increases the solvent capacity, the result being 88.4 per cent solution of the stone on the addition of 1 per cent sodium cholate. The addition of cholesterol to such a solution cuts down markedly the solvent action, as might be expected. These experiments are not quantitatively correct, as in some of them the fatty acid or cholesterol was not entirely in solution, but only in suspension. However, as qualitative experiments, the results are definite.

In experiments running for a 5 day period, the same type of conclusions may be drawn. Here again the results cannot be quantitatively carried from one set of experiments to another, because of the varying conditions under which they have been performed. Though as many conditions as possible were controlled, it is impossible to control all, such as the factor of the concentration of bile, the insurance of complete solution of substances tested and the like. The rate of solution of any one set of stones or even of the same stone varies probably because of the fact that even though stones from the same gallbladder are of essentially the same chemical composition, their structure may vary; that is, some may be more compact than others, or some may have a looser protein framework than others, or there may be more protein in one layer than in another. This would tend to alter the degree of solubility of the stones (table 7).

In a five day period (table 6), bile from a dog's gallbladder dissolved an average of 19 per cent of the stones. When bile from a dog's gallbladder was dialyzed 100 hours against tap water and then evaporated to the original volume, it dissolved significantly less of the stone. This means that the dissolving principle or complex is dialyzable and water-soluble. A dialysis of forty-eight hours had little effect on the solvent action of the bile, which indicates that uncombined bile salts are not responsible, since they readily dialyze.

Oleic acid in a 1 per cent suspension in p_H 8 buffer dissolved about 6.7 per cent of the stone in five days, whereas linseed oil soaps in 1 per cent concentration in water having an actual p_H of about 9 dissolved about 40.8 per cent of the stone. Since most of the fat at this p_H is present as soap, one must conclude also that soaps have some solvent capacity. This confirms the report of Brockbank.[30]

Lecithin in a 1 per cent solution had practically no solvent action.

Bile salts in a 6 per cent concentration had a solvent capacity, the cholate being more effective than the taurocholate, and neither being as good as dog's bile. Ten per cent sodium taurocholate in water dissolved about 5.8 per cent in five days. Since the sodium taurocholate used was impure, an attempt was made to purify the salt further by using standard methods. These steps, however, did not significantly alter the solvent capacity.

The addition of 1 per cent oleic acid to the bile salt solutions gave slightly higher rates of solution than did either the oleic acid or the bile salt alone. These results, while not marked, in the light of evidence to be presented later, may be of some significance.

The addition of lecithin in a 1 per cent concentration to bile salt and to mixtures of bile salt and oleic acid tends to lower somewhat their solvent capacity. This may possibly be explained on the basis of Spranger's [36] observations which show that an antagonism exists between lecithin and the solubility of cholesterol in colloidal solutions.

TABLE 7.—*Data Showing That the Rate of Solution Depends to Some Extent on the Stone**

Stone	Original Weight, Mg.	Days Required for Complete Solution	Average Loss per Day, Mg.
1L	0.830	34	24
2L	0.962	35	27
1M	1.182	38	31
2M	1.232	40	30
1P	1.153	27	43
2P	1.512	39	39
1Q	0.924	38	24
2Q†	1.085	35	31*

* Experiments in vitro were carried out with 1 per cent lauric acid in a p_H 8 buffered solution and the solution was changed daily and was used in 10 cc. amounts. The stones were weighed daily. Letters indicate the patients from whom the stones were obtained.

† Some fragmentation occurred which could not be checked by weight. This accounts for the discrepancy.

To determine the effect of agitation and daily replacement of the solvent on the stones, the following experiment was done. The stones were prepared as usual, but the solutions were not changed daily or were not agitated. The results of these experiments showed that the replenishing of the solvent at daily intervals increases the rate of solution, as might be expected. Without agitation, the rate of solution was definitely decreased, because agitation assures a uniform mixture of solvent and stone constituents, and washes away continually the surface layer in contact with the stone.

It was reported previously [17] that the rate of solution depends to a certain extent on the type of stone. The data are shown in table 7. The stones were obtained from different patients, and therefore were possibly of somewhat different composition. They were shaken in a 1 per cent lauric acid solution in p_H 8 buffer, the solution being renewed daily. The stones were weighed daily until they had practically entirely dissolved. The loss of weight of the stones was fairly uniform throughout the experiment. The rate of solution varied somewhat, being dependent on the source of the stones. The "L" stones, for instance, lost about 25 mg. daily, whereas the "P" stones lost about 41 mg. daily. If the weight of the stone from

36. Spranger: Biochem. Ztschr. **208**:164, 1929.

day to day is plotted against time, the curve is generally hyperbolic in type; that is, at the beginning, the rate of solution is greater than it is later. However, some stones may decrease in weight and then increase for a few days, then decrease again. The increase in weight is due to the swelling of protein, which can be readily seen by direct vision.

Purified cholesterol from gallstones was pressed into tablets, and these were used instead of gallstones in some experiments. The results are shown in table 8. It is seen that the rate of solution is essentially the same as that of gallstones in similar solutions. It is probable, therefore, that the cholesterol part of the gallstone is dissolved and leaves little or no support for the other constituents of the gallstone. This accounts for the early pitting or "moth eaten" appearance of the stones and for the crumbling which occurs later.

Since most of the fatty derivatives used in the experiments in vitro acted as good gallstone solvents, it was thought that perhaps an analysis of the lipoidal elements of man's bile and dog's bile might have some bearing on the question of why the latter dissolves and the former does not. The bile was analyzed by a modification of the Kumigawa-Sato[37] method suggested by Dr. Bloor in a private communication. This method, with further modifications, will be published later

TABLE 8.—*Effect of Shaking Cholesterol Pellets in Various Solutions at 37 C. for Five Days* *

Solution	Number of Experiment	Original Weight, Mg.	Loss in Weight, Mg.	Loss, per Cent
Bile from a dog's gallbladder	2	0.764	0.217	—28.4
0% sodium cholate in p_H 8 buffer	2	0.548	0.062	—11.3
0% sodium taurocholate in p_H 8 buffer	4	0.452	0.027	— 5.8
1% oleic acid in p_H 8 buffer	2	0.450	0.041	— 9.0
1% linseed oil soaps in water	2	0.391	0.202	—51.8

* The solutions were changed daily; 10 cc. quantities were used.

by Dr. K. K. Jones. The results of the earlier work have been reported by Walsh and Ivy.[17] It was shown that man's bile had a relatively high content of nonsaponifiable (chiefly cholesterol) matter (an average of 0.586 Gm. per hundred cubic centimeters of bile from gallbladders and of 0.171 Gm. per hundred cubic centimeters of bile from fistulas). The values for bile from human fistulas may be higher than the values for hepatic bile, because the samples were obtained from persons on whom cholecystostomy had been done, and it is reported that mere passage of bile through the gallbladder may result in the concentration of it from two to four times. The saponifiable matter of human bile was found to be about 1.284 Gm. per hundred cubic centimeters of bile from gallbladders and 0.520 Gm. per hundred cubic centimeters of bile from fistulas. This gives a ratio of nonsaponifiable to saponifiable matter of about 1:2. Dog's bile gave a ratio of about 1:20 in most cases, the nonsaponifiable matter being on the average 0.162 Gm. per hundred cubic centimeters of bile from gallbladders and 0.020 Gm. for bile from fistulas. The saponifiable matter in bile from gallbladders was about 4 per cent, while in bile from fistulas it was 0.3 per cent. This difference in the ratio of nonsaponifiable to saponifiable matter between bile of the human gallbladder and that of the dog's gallbladder, I believe, explains their difference in solvent action on human gallstones, since there exists no such outstanding difference between the two biles in concentration of bile salt. That the fatty content of bile alone is not the sole factor

37. Kumigawa-Sato: Biochem. Ztschr. **8**: 212, 1908.

in causing solution of stones is indicated by the fact that the addition of bile salts to a fatty acid emulsion enhances somewhat its solvent power. This is shown more directly in the following experiments.

Oleic acid alone dissolves solid cholesterol, but does not form a water-soluble compound of it. Oleic acid alone does not clarify an aqueous emulsion of cholesterol. Sodium cholate clarifies an emulsion of cholesterol of about 0.5 per cent strength, when the sodium cholate is in a concentration of about 20 per cent. For example, a mixture of 0.5 per cent cholesterol emulsion and 1 per cent oleic acid emulsion does not result in a clear, transparent solution. The addition of sodium cholate to this mixture so that a final concentration of about 20 per cent results clarifies it. A mixture of 0.5 per cent cholesterol emulsion and 6 per cent sodium cholate clarifies, if the oleic acid concentration is about 6 per cent or above. If the oleic acid concentration is increased markedly, the lipoids may not be entirely soluble, and on standing for several days, crystals (composition not determined) settle out. A mixture of 0.5 per cent cholesterol emulsion, 6 per cent sodium cholate and 9 per cent oleic acid yields a clear, transparent solution, which remains clear. On standing several days, a few crystals (plates and needles) settle out. On dialyzing this mixture, crystals form more rapidly.

It is probable that choleinic acid derivatives (choleinic acid being a complex of bile acid and fatty acid), which have been studied by Wieland and Sorge,[38] are responsible for clarifying the cholesterol emulsion. However, it is well known (Verzar[39]) that bile salts increase the solubility of fatty acids in aqueous solution. I believe that the fatty acids in turn render it possible for cholesterol to be held in aqueous solution, since certain fatty acids are better solvents of cholesterol than bile salts. According to this view, in the absence of bile salts, the fatty acids do not remain in aqueous solution, and hence cholesterol is not held in aqueous solution. This theory differs from that offered by Andrews, Schoenheimer and Hrdina[7] only in that I attach considerable importance to the solvent action that certain fatty acids have for cholesterol. Both theories are analogous in holding that an inadequate secretion of bile salts in the bile (when the bile is acid in reaction, as it is in the gallbladder) or a rapid absorption of bile salts by the mucosa of the diseased gallbladder leads to a precipitation of cholesterol; also, in holding that if the cholesterol elimination in the bile is increased and the bile salt elimination decreased, on the acidification of such bile in the gallbladder, which normally occurs, cholesterol is precipitated because of insufficient bile salts or acids to hold the fatty acids and cholesterol in solution.

This view is supported by the experiments cited on dialysis of bile. When bile from a dog's gallbladder was dialyzed 100 hours against tap water and then reduced to its original volume, the solvent action of the bile so treated on gallstones was reduced markedly. Dialysis for forty-eight hours had little effect on the solvent action. This may be explained on the basis of the work of Verzar,[39] who maintained that dialysis of complexes of bile salt and fat in the intestinal tract is the mode of absorption of fatty material when the intestinal contents are acid. The complexes of bile salt and fat, being large molecular aggregates, dialyze more slowly than smaller molecules or bile salts. Thus considerably longer time is required to remove these complexes from solution by dialysis than if the bile salt molecule itself were removed by dialysis. All the evidence that I have accumulated indicates to me that bile salts are not the sole factor concerned in the solvent action

38. Wieland and Sorge: Ztschr. f. physiol. Chem. **97**:1976, 1919.
39. Verzar: Klin. Wchnschr. **10**:1, 1931.

of the bile of the dog's gallbladder on human gallstones, and that the solvent action is due to the combined action of bile salts and fatty acids.

AN ATTEMPT TO PRODUCE GALLSTONES BY PARENTERAL ADMINISTRATION OF CHOLESTEROL IN RABBITS

There have appeared in the literature reports of the formation of cholesterol stones in the gallbladders of animals as the result of parenteral administration of cholesterol. Hansen [40] gave a good review of the literature on this subject. Dewey [41] and Badille [42] reported that they found cholesterol concrements after administration of cholesterol parenterally. Aoyma [43] and also Hansen [40] reported stone formation when a stricture was placed about the cystic duct. However, with no stricture of the cystic duct Hansen [40] found no concrements and only a few cholesterol crystals in the bile. Iwanga,[44] Engel and Cserna,[45] Stern [46] and Hospers [47] did not observe concretions on injections of varying amounts of cholesterol.

This work was repeated. Cholesterol emulsions were made by the method described by Dewey [41] in a 1 to 2 per cent concentration. The injections were given intravenously or intraperitoneally every other day for a period of from 35 to 107 days. Five rabbits received the emulsion intravenously, the total amount of cholesterol given these rabbits varying from 1.372 to 3.856 Gm. Two rabbits received the emulsion intraperitoneally for 51 and 55 days. One received a total of 3.166 Gm. of cholesterol and the other 2.917 Gm. Sodium chloride solution was administered intravenously and intraperitoneally to four rabbits which served as controls. In no case were concrements or cholesterol crystals found in the gallbladder. No marked changes were noted in the various organs mentioned by these workers. The only significant finding was a fatty infiltration of the liver of some of the animals given injections of the cholesterol emulsion.

CONCLUSIONS

The solution of human gallstones of the mixed cholesterol variety when placed in the gallbladder of the dog is dependent solely on the composition of the dog's bile.

The marked difference between man's and dog's bile in the ratio of the nonsaponifiable (cholesterol) to the saponifiable (fats and fatty acids) substances is the most probable explanation of why the bile of the dog's gallbladder dissolves human gallstones; it is not the difference in the normal cholesterol or bile salt content. Bile salts or bile acids either by holding the fats and fatty acids in aqueous solution at an acid p_H (the bile of the gallbladder being slightly acid) or by

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40. Hansen: Acta chir. Scandinav. **62**:483, 1927.
41. Dewey: Arch. Int. Med. **17**:757, 1916.
42. Badille, quoted by Hansen.[40]
43. Aoyma: Deutsche Ztschr. f. Chir. **132**:234, 1914.
44. Iwanga, quoted by Hansen.[40]
45. Engel and Cserna: Wien. klin. Wchnschr. **38**:123, 1925.
46. Stern: Arch. f. exper. Path. u. Pharmakol. **112**:129, 1926.
47. Hospers: Arch. Path. **14**:66, 1932.

combining with fatty acid to form choleinic acid render it possible for cholesterol to be held in aqueous solution. The solubility of cholesterol in bile depends on an interrelationship between fatty substances, bile salts or acids and cholesterol. Altering the concentration of any one of these alters the ability of bile to hold cholesterol in aqueous solution.

The rate of solution of the human gallstone in the dog's gallbladder is not altered significantly by adding either olive oil, coconut oil, lauric acid or foods high in cholesterol to the diet. The rate of solution of the stone is increased slightly by adding bile salt and cod liver oil to the diet.

Any factor which diminishes the flow of bile into and out of the gallbladder, or which diminishes the concentrating function of the gallbladder decreases the rate of solution.

Biliary stasis on the introduction of a stone into the normal gallbladder of a dog in some instances causes chronic cholecystitis and, in some instances, marked hyperplasia of the lymphoid follicles of gallbladder.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc.—Brompt, professor of parasitology at the Faculté de Médecine, Paris, has contracted Rocky Mountain spotted fever while working with guinea-pigs infected with the disease.

Leon C. Havens, director of the bureau of laboratories of the State Department of Health of Alabama since 1922, has died.

At the new Leonard Wood Laboratory for the eradication of leprosy in the Philippines, investigative work is planned in the near future by Malcom H. Soule, University of Michigan, and E. B. McKinley, George Washington University.

Society News.—The newly elected officers of the Society for Experimental Biology and Medicine are: president, A. R. Dochez; vice-president, E. L. Opie, and secretary-treasurer, A. J. Goldforb.

The American Society for Experimental Pathology elected the following officers at its meeting held in Cincinnati, April 10, 11 and 12, 1933: president, Carl V. Weller; vice-president, S. Burt Wolbach, and secretary-treasurer, C. Phillip Miller.

The Pathological Society of Eastern New York has recently been organized. The membership consists of pathologists from Albany, Troy, Schenectady, Amsterdam, Gloversville, Glens Falls, Cooperstown, Mount McGregor, and Pittsfield, Mass. The secretary is Dr. Arthur W. Wright, Bender Hygienic Laboratory, Albany, N. Y.

Medical Fellowship Board of the National Research Council.—According to *Science*, eighteen fellowships in medicine, including two renewals, were awarded at the spring meeting of the Board. The following are the appointees: Caroline C. Bedell, Maurice Brodie, James K. W. Ferguson, Earl W. Flosdorf, Louis S. Goodman, William G. Gordon, Arthur T. Hertig, Joseph Hughes (renewal), Thomas H. Jukes, James M. Orten, Alwin M. Pappenheimer, Wesley T. Pommerenke, Clarence F. Schmidt, Jr. (renewal), Henry G. Schwartz, Randall L. Thompson, William McC. Tuttle, Arthur J. Vorwald and Samuel J. Weinberg. The next meeting of the Board will be held in September, 1933, and applications to be considered at that time should be sent to 2101 Constitution Avenue, Washington, D. C., on or before August 1.

Cancer Review.—The British Empire Cancer Campaign has ceased publishing this journal on the completion of volume 7, 1932.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

INCIDENCE AND DISTRIBUTION OF THE AGE OF DEATH IN RHEUMATIC HEART DISEASE. DAVID DAVIS and SOMA WEISS, Am. Heart J. **8**:182, 1932.

The incidence and distribution by decades of the age of death in 5,060 consecutive necropsies in the Boston City Hospital have been analyzed. Deaths from rheumatic heart disease in 229 cases were distributed widely from the second to the fifth decades of life inclusive, the largest number of deaths occurring in the fourth or fifth decades. Of 47 patients in whom subacute bacterial endocarditis was superimposed on an old rheumatic heart disease, 40 died before the age of 50. In all of the 19 cases of malignant endocarditis superimposed on rheumatic heart disease, death occurred before the age of 40. It is to be emphasized that as late as the sixth and seventh decades of life rheumatic heart disease is frequently present in patients with cardiac symptoms and signs. The evidence presented suggests that the age distribution of death in the hospital population studied is roughly parallel to that in the population at large. An estimate of the expected frequency of fatal rheumatic heart disease among the living population of the state of Massachusetts is presented. This estimated frequency of fatal rheumatic heart disease must, however, represent but a small portion of the total prevalence of rheumatic heart disease in the living population.

AUTHORS' SUMMARY.

THE RELATIONSHIP OF CALCIUM METABOLISM TO DISEASES OF BONE. D. P. BARR, Am. J. Cancer **16**:1424, 1932.

Decalcification of the skeleton may arise from diverse causes. It has been observed in association with disuse, as in arthritis, fractures and other conditions enforcing prolonged and extreme inactivity. It is seen in rickets, hunger and war osteopathies, osteomalacia and celiac rickets, conditions which are all apparently dependent on a lack or loss of vitamin D and lack of sunlight, and more or less readily controlled by supplying these missing factors. It is also seen in association with increased function of the parathyroid glands, both experimentally following the injection of parathyroid extract, and in the clinical condition of osteitis fibrosa cystica, the most effective treatment of which is removal of abnormal parathyroid tissue. The objective finding of decalcification must be carefully differentiated. The terms osteomalacia and osteoporosis, while perhaps sufficiently exact at the time they were devised, have been used as catch baskets for a great variety of obscure clinical conditions and are not helpful in the functional explanation of differences in bone texture. Physiologic considerations in the differentiation of decalcification not only are essential to proper diagnosis but are of the greatest importance in treatment. The comparatively rare diseases in which there is too great calcification in bones have been insufficiently studied. Although there is in Paget's disease and in osteosclerosis much indirect evidence of disturbances in calcium metabolism, the nature of these abnormalities is not clearly understood.

AUTHOR'S SUMMARY.

CAUSES OF DEATH IN STATUS LYMPHATICUS. EDWARD H. CAMPBELL, Am. J. Dis. Child. **44**:1297, 1932.

Many theories have been advanced to explain the cause of death in cases of status lymphaticus. Only three of these are worthy of consideration; (*a*) suffocation from the pressure of an enlarged thymus on the trachea or nerve trunks in

the mediastinum, (*b*) apoplexy from the rupture of a hypoplastic cerebral vessel and (*c*) heart failure due to an insufficiency of the suprarenal hormones. The last of these is by far the most important. It explains the sudden death as due to the chemical and organic changes induced by the insufficiency of the cortical and medullary hormones from the suprarenal glands. The chief chemical factors concerned in sudden heart failure are those that cause a disturbance in the chemical properties and electrical reactions of cells that are assumed to be the important factors in the initiation, propagation and maintenance of the heart beat. In cases of status lymphaticus there is a deficiency of some of the important chemical elements that normally tend to establish contractility of the cardiac muscle, and this deficiency so alters the composition of muscle tissues and fluids that the electrical stimulus for muscle contraction may suddenly become lost, resulting in stoppage of the heart.

AUTHOR'S SUMMARY.

CONVERSION OF LEVULOSE TO DEXTROSE DURING ABSORPTION FROM THE INTESTINE. G. E. BURGET, P. MOORE and R. LLOYD, Am. J. Physiol. **101**: 570, 1932.

Experiments conducted with closed loops of intestine in dogs and isolated living segments of rabbit intestine and examination of the blood from the mesenteric vein in laparotomized dogs after the administration of levulose showed absorption of the levulose as such, without conversion to dextrose.

H. E. EGGERS.

TRANSPLANTATION OF ADRENAL CORTICAL TISSUE IN THE RAT. L. C. WYMAN and C. TUM SUDEN, Am. J. Physiol. **101**:662, 1932.

The effects of autotransplantation and homotransplantation of suprarenal cortex were studied in rats. Autoplastic transplants grew more extensively in female than in male rats, with the total amounts of regenerated tissue approximately constant for each sex. Homoplastic transplants were also successful, even with accompanying autotransplantation; heteroplastic transfer was uniformly unsuccessful. From the fact that all implantations were unsuccessful in animals with remains of the glands themselves, it would appear that there is a physiologic factor limiting the growth of this tissue, and that hyperfunction cannot be induced by implantation methods.

H. E. EGGERS.

STUDIES OF LIVER FUNCTION. T. W. PRATT, H. W. VANLANDINGHAM, E. E. TALLEY, J. M. NELSON and E. O. JOHNSON, Am. J. Physiol. **102**:148, 1932.

These writers confirm the findings of Rosenthal on the value of bromsulphalein injected intravenously as a delicate and effective method for the study of liver function. In dogs, after complete blockage of the hepatic circulation and union of the portal vein with the vena cava, there is a high retention of the dye. In dogs, an effective basis for physiologic study of the liver was obtained by taking samples of blood three and ten minutes after the injection.

H. E. EGGERS.

THE STIMULATION OF MUSCLE RESPIRATION BY CARBON MONOXIDE. W. O. FENN and D. M. COBB, Am. J. Physiol. **102**:379 and 393, 1932.

The respiration of frog muscle in oxygen diluted with carbon monoxide is from 1.5 to 3 times greater than that observed in equivalent concentrations of oxygen in pure nitrogen. The respiratory quotient of frog muscle in oxygen diluted with carbon monoxide is 0.76, as compared with the value of 0.87 observed in air, a difference which may have its origin in an actual burning, by the muscle, of carbon monoxide to form carbon dioxide. The effect is manifested also by the heart and skeletal muscles of the rat and, to a considerably smaller extent, by rat liver, spleen, nerve, testis and spinal cord and by the Jensen rat sarcoma.

(The inhibiting effect of carbon monoxide on the respiration of the organism as a whole may be the result, specifically, of the blockade by that gas of the oxygen-carrying, storing and soluble-rendering powers of hemoglobin and its intracellular analog, cytochrome. The Warburg postulate that cellular respiration is motivated by an oxygen-activating, carbon monoxide-susceptible "respiration ferment" was based on observations of the respiration of cells rich in cytochrome. It may be incorrect. Shibata and Tamiya recently reported the preparation of cytochrome-free cultures of an aerobic mold which respire as effectively in the presence of carbon monoxide as in its absence.)

ARTHUR LOCKE.

EXPERIMENTAL EDEMA FROM PLASMA PROTEIN DEPLETION. MICHAEL J. LEPORE and AUGUSTA B. MCCOORD, Arch. Int. Med. **50**:488, 1932.

A study was made of the water and chloride metabolism of normal dogs and of dogs that had been rendered hypoproteinic by plasmapheresis. The sites (suprarenal, intestine, kidneys, liver, lung, pancreas, spleen, muscle and skin) of deposition of water and chloride were ascertained by analysis of tissue. On the basis of the data obtained, it is concluded that the edema that occurs in dogs rendered hypoproteinic by plasmapheresis is a sodium chloride edema, the development of which can be hastened by increasing the intake of fluid and sodium chloride of these animals.

AUTHOR'S SUMMARY.

MATURING EFFECT OF ROENTGEN RAYS ON BLOOD-FORMING CELLS. RAPHAEL ISAACS, Arch. Int. Med. **50**:836, 1932.

The effect of roentgen irradiation, in both small and large doses, on developing blood cells is stimulating, the result depending on the stage of development of the cells. Cells in the myeloblastic, lymphoblastic or a younger stage are stimulated to rapid reproduction, because of their growth potentiality at that stage, whereas cells in the myelocytic or the medium-sized lymphocytic stage are stimulated to grow through the rest of their life in an orderly manner, because normally these cells do not divide and multiply. They die or are eliminated as normal, senile cells. There is no evidence of a toxic necrotic action of the roentgen rays when they are applied in therapeutic doses. The process is hastened by larger doses, so that senility is reached more quickly, giving the impression of a "depressing" action. The period between the application of the roentgen rays and the time when the cells reach senility is the latent period. This corresponds to the time necessary for the cells to mature. The action of roentgen rays on living, blood-forming cells is one of stimulation to divide or to mature in a normal manner and not one of toxic necrosis. The tissue cells which have a long adult life (muscle cells, nerve cells and fibrous tissue cells) appear resistant to roentgen rays, whereas those that have a short adult life (germ cells and leukocytes) die soon after they are "stimulated" to develop to senility. They all have the same susceptibility, but death follows at varying intervals, depending on how long the cells normally survive in their physiologic life.

AUTHOR'S SUMMARY.

EFFECT OF EXPERIMENTAL HEPATIC DAMAGE ON THE HEMATO-ENCEPHALIC BARRIER. C. WESLEY EISELE and LATHAN A. CRANDALL, Arch. Neurol. & Psychiat. **28**:1383, 1932.

The authors studied the hepato-encephalic barrier by using intravenous injections of bromosulphalein, phenosulphonphthalein, neutral red and potassium iodide and the bromide method of Walter. They also determined the inorganic phosphorus of the cerebrospinal fluid. Prior to the injection of the substance a cisternal puncture was made and the needle was left in situ, while samples were taken over a period of from one to three hours. Normal dogs and dogs with an Eck fistula were used. The permeability quotient of the meninges was determined by Walter's

bromide method. While the authors do not deny the possible effect of damage to the liver on the cerebrospinal system, the conclusion they arrived at was that the hepatic damage does not affect the barrier function.

G. B. HASSIN.

PATHOGENESIS OF THE "STRAWBERRY" GALLBLADDER. R. ELMAN and E. A. GRAHAM, Arch. Surg. **24**:14, 1932.

Clinical and anatomic observations supported by experiment suggest that the initiation of the deposit of cholesterol in the mucosa of the gallbladder is infection. The authors offer experimental evidence that cholesterol is secreted by the mucosa of the gallbladder rather than absorbed by it. The ability of the mucosa to secrete cholesterol infection of the wall of the gallbladder are the two factors responsible for the so-called "strawberry" gallbladder or cholesterosis. Analysis of the bile in a gallbladder isolated by ligature of the cystic duct showed an increase of from 18 to 520 per cent in cholesterol. The cholesterol output in liver bile as compared with gallbladder bile showed a concentration from 18 to 153 per cent greater in the latter. So-called white bile obtained from the bile ducts and gallbladder contained cholesterol, indicating that the cholesterol not only was not absorbed, but also had probably been secreted.

N. ENZER.

THE EFFECT OF VIOSTEROL ON THE PERIOSTEUM AND EXPERIMENTAL FRACTURE. R. C. GRAUER, Arch. Surg. **25**:1035, 1932.

Experiments led to the conclusion that viosterol stimulates the osteogenic layer of the periosteum. If the dosage is within therapeutic range, osteogenic differentiation results. Overdosage, however, causes a proliferation of the fibrous layer of the periosteum and decalcification of bone and retards repair. In many cases an overdosage of viosterol produces a lesion very similar to fibrous osteitis.

N. ENZER.

ACUTE GLOMERULITIS FOLLOWING INJECTION OF STREPTOCOCCUS VIRIDANS INTO THE RENAL ARTERY. N. MCLEOD and G. G. FINNEY, Bull. Johns Hopkins Hosp. **51**:300, 1932.

By the use of certain strains of green streptococci one may produce glomerular, tubular and interstitial changes in rabbits which simulate in some respects glomerular or hemorrhagic nephritis in man. Different strains of green streptococci differ markedly in their power to produce renal lesions. The lesions produced are even more extensive than those previously obtained with B-hemolytic streptococci. In general, lesions were found somewhat more frequently in sensitized than in normal animals, though within the former group the degree of allergy, as estimated by cutaneous and ocular reactivity, apparently bears no relation to the development of renal lesions.

AUTHORS' SUMMARY.

JAKE PARALYSIS. PARALYSIS FOLLOWING THE INGESTION OF JAMAICA GINGER EXTRACT ADULTERATED WITH TRI-ORTHO-CRESYL PHOSPHATE. J. G. KIDD and O. R. LANGWORTHY, Bull. Johns Hopkins Hosp. **52**:39, 1933.

This paper reviews recent observations on an epidemic of polyneuritis which followed the ingestion of Jamaica ginger extract adulterated with tri-o-cresyl phosphate, in 1930 and 1931. The authors injected the pure tri-o-cresyl phosphate subcutaneously into cats and produced a marked paralysis in every case in which the dosage of the drug was adequate. There was always a definite latent period before the symptoms appeared. In the cats used, as in man, the caudal portion of the body was affected first and most severely, and the paralysis then spread cephalward. By using divided doses of the drug given at fairly long

intervals, it became evident that the effect of the drug was cumulative and that it was eliminated slowly, if at all. Further, the degree of paralysis varied directly, and the length of the latent period indirectly, with the amount of the poison given. If large doses of the purified tri-o-cresyl phosphate were injected the cats were suddenly seized with a rapidly fatal, flaccid paralysis exactly comparable to that described by Landry in 1859. Pathologic studies revealed lesions of the ventral roots of the spinal cord and of the somatic motor cells of the anterior horns. The autonomic cells were less severely injured. In a cat which survived for six weeks there was some injury of the large cells of the dorsal ganglions and fibers of the dorsal roots.

AUTHORS' SUMMARY.

METHOD FOR PREDICTION OF SEX IN THE UNBORN. J. H. DORN and E. I. SUGARMAN, J. A. M. A. **99**:1659, 1932.

By injecting into rabbits, fully 3 months old, whose testicles were in the inguinal canal, urine from women between five and ten months pregnant, we were able to prognosticate the sex of the unborn child in eighty of eighty-five cases. The basis for our prognosis was the microscopic changes in the testicle of the rabbit forty-eight hours after injection. The testes of the rabbits given injections of urine from women carrying an unborn female child were developed precociously. The testes of the rabbits given injections of urine from women carrying an unborn male child were not so stimulated.

AUTHORS' SUMMARY.

THE PROTECTION OF INSULIN BY ANTIPROTEASES AND ITS ABSORPTION FROM THE INTESTINE. BEN K. HARNED and THOMAS P. NASH, JR., J. Biol. Chem. **97**:443, 1932.

Insulin administered by stomach tube to depancreatized dogs in combination with antitrypsin was found to cause a marked decrease in the excretion of sugar. The preparation of an antitrypsin, which was 90 per cent effective in preventing inactivation of insulin by pancreatin in vitro under conditions favorable to tryptic digestion, is described. The insulin and antitrypsin mixture was found to have no effect in the normal dog when introduced into the duodenum. It was found that insulin, alone or with antitrypsin, introduced into the duodenum of depancreatized dogs abolished the excretion of sugar for short periods. Antitrypsin alone was found to have no effect on the excretion of sugar. The absorption of insulin directly from the intestine was small, and was not improved by the presence of the antitrypsin.

L. E. SHINN.

EFFECT OF SHORT WAVE FEVERS ON RABBITS. R. A. BOAK, C. M. CARPENTER and S. L. WARREN, J. Exper. Med. **56**:725, 1932.

The repeated elevation of the body temperatures of male and female rabbits to 41 and 42 C. (105.8 and 107.6 F.) from two to five times a week by short radio waves (30 m.), beginning between 29 and 171 days of age and extending through the first period of gestation, failed to injure their growth or to interfere with mating, fertilization or the development of young in utero. Litter mates were kept for controls. The rabbits exposed to the short waves showed, in the majority of cases, a greater percentage gain in weight than did the controls. The kindling age of the treated group was, on an average, 2 weeks above that of the nontreated group. The fever-treated females averaged five and one-half young per litter with an average weight of 41 Gm., while the untreated females averaged seven per litter with an average weight of 36.5 Gm. The repeated elevation of body temperature by short radio waves is a safe procedure when temperatures no greater than those within physiologic limits are employed.

AUTHORS' SUMMARY.

EXPERIMENTAL OSTEOPOROSIS AND FIBROUS OSTITIS IN DOGS. H. L. JAFFE, A. BODANSKY and J. P. CHANDLER, J. Exper. Med. **56**:823, 1932.

In all age groups the effects of the administration of ammonium chloride were found to be strikingly dependent on the calcium intake. Dogs receiving an adequate calcium diet and ammonium chloride showed less decalcification than those receiving a diet low in calcium with or without ammonium chloride. In the younger groups ammonium chloride added to a diet low in calcium brought out more striking changes than a diet of low calcium content alone. When the decalcification was less severe—in the oldest dogs on the diet low in calcium, with or without ammonium chloride, and in the younger dogs on an adequate calcium intake with ammonium chloride—generalized thinning of the bones without marrow fibrosis resulted (osteoporosis). When the decalcification was rapid and severe—in the youngest dogs on a diet low in calcium, particularly with ammonium chloride—generalized decalcification and secondary marrow fibrosis resulted (osteitis fibrosa). Generalized osteitis fibrosa is a rather inclusive term and may be applied to the histologic picture which results when clinical or experimental decalcification is rapid and therefore leads to extensive marrow fibrosis. The special underlying causes of the decalcification may incidentally contribute features to modify the generalized osteoporosis or osteitis fibrosa, as in rickets and in Recklinghausen's disease.

AUTHORS' SUMMARY.

STUDIES ON MOUSE LEUKEMIA. M. N. RICHTER and E. C. MACDOWELL, J. Exper. Med. **57**:1, 1933.

The results indicate that leukemia has not been transmitted by inoculation without the introduction of living cells. The interval between inoculation and death bears an inverse relationship to the number of living cells inoculated.

AUTHORS' SUMMARY.

THE ROLE OF THE SPLEEN IN IRON METABOLISM. E. D. WILSON and E. B. KRUMBHAAR, J. Exper. Med. **57**:65, 1933.

In normal dogs on a diet adequate in iron, removal of the spleen brings about a temporary increased loss of iron from the body; this is most marked during the period of developing anemia. It is possible that the anemia following splenectomy is the cause of the negative balance, though it is more probable that splenectomy removes an important iron depot and that this loss of iron is the cause of the anemia. Further investigation of anemia following splenectomy is needed, however, before final conclusions can be drawn as to the mode of its production.

AUTHORS' SUMMARY.

THE OCCURRENCE OF ANTIGOITROUS SUBSTANCES IN PLANTS. D. MARINE and associates, J. Exper. Med. **57**:121, 1933.

The development of simple goiter in rabbits, which results when they are fed on a diet consisting chiefly of cabbage (or other Brassicae), can be prevented by the feeding of certain fresh plants. Lawn grass, fresh alfalfa, skunk cabbage and steamed cabbage press juice have been found effective. Washing Brassicae with water improves their goiter-producing action, probably by the removal of goiter-preventing substances. The antigoitrous effect of plant juices was found to vary with the amount of reducing substance other than glutathione present. Skunk cabbage press juice, obtained from growing plants during the spring months, contained a large amount of iodine-absorbing material, and was effective in causing involution of hyperplastic glands, while juice obtained from mature plants in July, when the iodine-absorbing material had nearly all disappeared, was quite ineffective. The parenteral administration of certain extracts from cabbage, containing the reducing material, also had a marked involutional action on the

thyroid. These extracts were practically iodine-free. The substance or substances believed to be responsible are easily soluble in water, ethyl alcohol and aqueous ethyl alcohol, and are precipitable by lead acetate at a p_H of from 0.68 to 0.70. They are partially destroyed by exposure to air and by steam at 100 C. Hexuronic acid isolated by Szent-Györgyi from cabbage, orange juice and suprarenal glands may be the antigoitrous agent. It cannot be glutathione.

AUTHORS' SUMMARY.

REACTIONS TO BLOOD TRANSFUSION. S. H. POLAYES and M. LEDERER, J. Lab. & Clin. Med. **17**:1029, 1932.

This report is based on observations on 2,500 transfusions and a review of the literature. There are 132 references to the literature at the end of the article. The various causes of reactions to blood transfusion are considered under the following headings: incompatibility between the blood of the donor and the blood of the recipient, the use of unclean apparatus, the use of citrate solutions, incipient coagulative changes in the transfused blood, allergic phenomena in the recipient, systemic diseases in the recipient and transmission of disease. The danger of transmission of disease is illustrated by a number of observations. The transmission of asthma, measles, smallpox, malaria and syphilis has been reported. It is known that syphilis has been transmitted to the donor.

Pathologic Anatomy

SYPHILITIC PLASTIC LINITIS. H. BRUNNER, Virchows Arch. f. path. Anat. **282**: 883, 1931.

Two years previous to her admission to the hospital, a woman, aged 36. had begun to have symptoms of progressive pyloric obstruction. The roentgenogram showed a typical leather bottle stomach. She had a unilateral Argyll Robertson pupil and serologic reactions strongly positive for syphilis. The clinical diagnosis was plastic linitis of syphilitic origin. Because of the diffuse involvement, partial gastrectomy was peformed. The wall of the pyloric and prepyloric portion of the stomach was diffusely greatly thickened, the increase being chiefly of the submucous coat. The mucosa was intact but atrophic. The perivascular reaction in the thickened submucosa warranted a histologic diagnosis of syphilis. Spirochetes could not be demonstrated in the tissue. Operation and postoperative antisyphilitic therapy were followed by rapid gain in weight and complete recovery.

O. T. SCHULTZ.

ACUTE HEMORRHAGIC MYOSITIS. E. DEÁK, Virchows Arch. f. path. Anat. **282**: 895, 1931.

According to Deák, acute hemorrhagic myositis is rare. He reports five cases occurring in adults, who died of acute infection with hemolytic streptococci. Four were cases of fatal sepsis with origin in the upper part of the respiratory tract. In two of these, the gluteal muscles were involved, and pressure and decubitus may have been factors. In two, the muscles of the calf and arm were involved. In the fifth case, phlegmonous inflammation of the tissues of the upper part of the arm followed a fracture of the neck of the humerus. The histologic features were degeneration and necrosis of the muscle fibers and separation of the latter by hemorrhagic infiltration of the connective tissue. Variable numbers of leukocytes were present.

O. T. SCHULTZ.

HEMATOLOGY OF ENDEMIC GOITER. S. I. SCHERMANN, Virchows Arch. f. path. Anat. **283**:1, 1932.

Three expeditions were sent from Kazan to study endemic goiter among the Cheremisses. The leukocytic blood picture was carefully studied in 610 of these

natives of the Tartar Republic. The total count was normal in 62 per cent, decreased in 18 per cent and increased in 20 per cent. Neutrophilic granulocytes with segmented nuclei were decreased in number, and cells with staff nuclei correspondingly increased, indicating a decided shift from normal. Eosinophilia was absent in only 2. Basophils, Türck's irritation forms and monocytes were increased. Lymphocytosis was noted in 30 per cent, and a decrease in the total number of neutrophilic granulocytes in 79 per cent. Schermann interprets the hematologic findings as characteristic of intoxication.

O. T. SCHULTZ.

CHOLESTEATOMATOUS CYST OF THE LOWER JAW. A. W. RYWKIND, Virchows Arch. f. path. Anat. **283**:13, 1932.

Cholesteatoma of the lower jaw is rare. That of the upper jaw is more frequent, but is usually the result of retention within a chronically inflamed maxillary sinus. The lesion described by Rywkind was a cyst about the size of a walnut situated in the lower jaw at the root of a tooth. It consisted of an outer wall of dense fibrous tissue and a lining about the cyst cavity of squamous epithelium. The cavity contained fatty material. The basal layer of the epithelium was hyperplastic in places. In such areas there were formed hyperplastic outgrowths of differentiated epithelium, which was completely or partly hornified. Lining epithelium was absent in areas and had been replaced by inflammatory granulation tissue that contained cholesterol crystals derived from the degenerated hornified epithelium.

O. T. SCHULTZ.

CONGENITAL ABSENCE OF PARATHYROID BODIES. R. RÖSSLE, Virchows Arch. f. path. Anat. **283**:41, 1932.

Following discussion of the more usual maldevelopments of branchiogenic origin, Rössle presents two examples of absence of the parathyroid bodies, as determined by serial sectioning of the tissues of the neck. One was found in an anencephalic infant who lived nine hours; the other, in a female infant with no external malformations who lived ten weeks. Symptoms began at the age of six weeks and were those of severe spasmophilia. Necropsy revealed the absence of the substernal portion of the thymus and of the isthmus of the thyroid gland. No parathyroid tissue could be found on serial sectioning of the cervical tissues. The second case offers proof that infantile tetany may be due to deficient parathyroid gland function.

O. T. SCHULTZ.

FETAL ERYTHROLEUKOBLASTOSIS WITH FATAL ICTERUS NEONATORUM. D. Y. KU and Y. LI, Virchows Arch. f. path. Anat. **283**:62, 1932.

The condition occurred in a full term, male, Chinese infant, who developed rapidly progressing icterus twelve hours after birth and died on the fifth day after birth. The first child of the mother became jaundiced on the fourth day of life and remained so for twenty days; the child lived. The second child became icteric on the third day and died on the fifth day after birth. The third child became jaundiced during the first day and died on the third day of life. Postmortem examination of these infants was not made. The fourth child, the subject of this report, revealed no abnormalities at necropsy, except jaundice. Histologic examination, however, revealed areas composed of young erythroblastic and myeloblastic cells in the liver, spleen, gastric wall and skin. The bone marrow contained similar cells. Ku and Li believe the condition to be a disease of the hematopoietic system identical with the erythroleukoblastosis associated with congenital anasarca. Whether edema or a fatal form of icterus develops may depend on the age of the fetus at the onset of the disease and perhaps also on factors as yet unknown.

O. T. SCHULTZ.

THE SPECIFIC HISTOLOGY OF THE INTERSTITIAL PNEUMONIA OF ACQUIRED SYPHILIS. A. ANTONOW, Virchows Arch. f. path. Anat. **283**:413, 1932.

Antonow briefly reviews the differences of opinion as to the specific histology of the interstitial pneumonia of acquired syphilis, forms with true gumma formation not being taken into consideration. He reports two cases, in which there was undoubted evidence of syphilis in organs other than the lung. In one case the upper left lobe was involved, and the process was early. The alveolar septums were thickened by slightly fibrillar cellular tissue, which took its origin from about the blood vessels. Small granulomatous nodules, which Antonow terms miliary syphilomas, projected from the septums into the alveolar spaces. In the second case, the upper and lower lobes of the right lung were involved, and the process was older. There was marked fibrosis, with no especial perivascular localization. Spirochetes could not be demonstrated in either lung; nor have they been demonstrated in other, previously reported cases. Antonow considers the changes seen in the first case histologically specific. The fibrotic changes in the second case, representing the late stage, are not characteristic; the probable syphilitic nature of the process in this stage must be deduced from the anatomic evidence of syphilis in other organs.

O. T. SCHULTZ.

LOCALIZED AMYLOIDOSIS WITH MACROGLOSSIA. G. GERSTEL, Virchows Arch. f. path. Anat. **283**:466, 1932.

In a woman, aged 52, who had always been well, there developed "over night" marked swelling and hardening of the gums and tongue. The swelling quickly reached its maximum intensity and persisted until death two years later. Thickening and hardening of the skin of the neck developed more gradually and continued progressively until death. The gross anatomic diagnosis was diffuse amyloidosis of the tongue, buccal tissues, upper part of the esophagus, intestine, and of the skin, subcutaneous tissues and muscles of the neck. Organs commonly involved in generalized amyloidosis, namely, the spleen, liver, kidneys, suprarenal glands and lymph nodes, contained no amyloid. The suprarenal glands and the lymph nodes of the mesentery and elsewhere were surrounded by amyloid, but the organs themselves were free from it. The gluteal muscles, the diaphragm and the heart muscle contained much amyloid. The amyloid material formed thick sheets or large nodular masses at the junction of submucosa and muscle, or of connective tissue and muscle, or of nerves and surrounding tissue. Thorough microscopic examination of the various tissues and organs of the body revealed no suppurative or inflammatory process to which the amyloidosis might be ascribed. Lubarsch, who has reported three cases, or noted as characteristic the occurrence in situations not usually involved in generalized amyloidosis, the absence of amyloid infiltration in organs usually involved in the generalized form, and the absence of any suppurative process or other underlying disease to which the condition might be ascribed. He also noted that the chemical reactions of localized amyloid are usually atypical. Common manifestations are: involvement of the skin over the infiltrated deeper tissues (amyloid pseudoscleroderma), involvement of voluntary musculature (amyloid pseudomyotonia) and tumor-like enlargement of the infiltrated organ or tissue (amyloid pseudotumor). Pick, from whose institute the present report comes, believes that these manifestations together with the congo red test should make clinical diagnosis of the condition possible.

O. T. SCHULTZ.

POSTMORTEM EXAMINATION OF THE BODIES OF THE SOVEREIGN BISHOPS OF WÜRZBURG DURING THE SEVENTEENTH AND EIGHTEENTH CENTURIES. A. HOLZMANN, Virchows Arch. f. path. Anat. **283**:513, 1932.

The examinations were made during the years 1684 to 1795. The bodies were those of the seventy-third to eighty-second (and next to last) princely bishops of Würzburg. The exenteration was done for the purpose of embalsaming or

embalming the bodies. A brief clinical résumé, prepared by Holzmann from the documents of the period, precedes the copies of the Latin protocols. Holzmann's translation into German follows, with a brief summary and interpretation. Most of the reverend gentlemen reached the ripe age when pneumonia becomes the "friend of the aged"; the youngest, aged 47 at the time of death, was a victim of typhoid. They were not immune to arteriosclerosis and prostatic hypertrophy, nor, if aortic aneurysm was due to syphilis then as it is now, to the Gallic disease. The pathologist of 1932 who begins his necropsy protocols with "This is the body of a fairly well nourished . . ." may be able to detect the ancestry of his opening phrase in these words at the beginning of a protocol of 1749: *"Ea est humani Corporis quam nobilis, tam fragilis conditio. . . ."*

O. T. SCHULTZ.

CHRONIC LEUKEMIC RETICULO-ENDOTHELIOSIS. C. BÖHNE and L. HUISMANS, Virchows Arch. f. path. Anat. **283**:575, 1932.

Of previously reported cases of reticulo-endotheliosis, Böhne and Huismans accept only five as examples of leukemic reticulo-endotheliosis or monocytic leukemia, which they define as a disease of the reticulo-endothelial system characterized by hyperplasia of this system, with resumption of hematopoietic activity and a persistent increase of monocytes in the peripheral blood. The total leukocytes need not be increased. Their patient was a woman 50 years old. The illness began two years before death with rather rapid enlargement of the left tonsil to the size of a hen's egg. Biopsy led to a diagnosis of lympho-epithelial tumor of the tonsil. At this time the blood contained 75 per cent granulocytes, 10 per cent lymphocytes and 15 per cent monocytes. Roentgen irradiation was followed by rapid decrease in the size of the tumor and by improvement in the general condition. In four weeks, however, the general condition was worse, the spleen was greatly and the liver moderately enlarged, and all the superficial lymph nodes were palpable. At this time the erythrocyte count was 1,800,000, hemoglobin 35 per cent, and leukocyte count 4,200, with 73 per cent granulocytes and 27 per cent monocytes that gave a negative oxidase reaction. Roentgen irradiation of the spleen reduced the monocytes to 3 per cent and the total leukocyte count to 1,800. Three days before death the leukocyte count was 1,800, with 68 per cent granulocytes, 10 per cent large lymphocytes, and 22 per cent monocytes. Necropsy revealed hyperplasia of all the lymph nodes, spleen and bone marrow and enlargement of the liver. The hyperplasia was due to proliferation of large mononuclear cells derived, according to the authors, from the reticulo-endothelial system and identical, they believe, with the monocytes of the peripheral blood. Similar cells were seen in the periportal tissue of the liver, in the interstitial tissue of the pancreas and kidney and in the connective tissue of the skin. The differences between leukemic reticulo-endotheliosis, as defined by the authors, and myeloblastic leukemia, aleukemic leukemia, infectious monocytosis and reticulo-endothelial hyperplasia with lipoid storage are discussed. (The name of one of the authors is spelled Huismans in the title of the article and Huismann in the table of contents and volume index.)

O. T. SCHULTZ.

THE STRUCTURE OF LYMPH NODES IN RELATION TO THEIR FUNCTION. H. KUBO, Virchows Arch. f. path. Anat. **283**:593, 1932.

To investigate the assumption that the function of lymph nodes varies with their location and with the different kinds of materials brought to them in different situations, and that such differences in function should be manifest in morphologic differences, Kubo made a comparative histologic study of the para-aortic and mesenteric nodes in 200 necropsies of persons ranging in age from birth to 80 years. The para-aortic nodes were selected because they have been termed hemolymph nodes by some investigators and have been believed to have an erythropoietic function. In 84.5 per cent of the necropsies the sinuses of the para-aortic nodes contained erythrocytes, as compared with only 19.5 per cent for the mesenteric

nodes. Iron pigment was noted in 49.4 per cent of the para-aortic nodes, and in only 3.5 per cent of the mesenteric nodes. Both red corpuscles and iron increased progressively with age in the para-aortic nodes. Kubo denies that the para-aortic nodes are true hemolymph nodes. The erythrocytes in their sinuses are brought to the nodes from other situations. Iron is deposited first in the sinus endothelium and later in the reticulum cells. The difference in localization of iron is dependent on time and not, as Nordmann had claimed, on function. Kubo denies the existence of hemolymph nodes in man.

O. T. SCHULTZ.

SYMMETRICAL HYPERTROPHY OF THE GUMS. H. A. KOBLIN, Virchows Arch. f. path. Anat. **283**:631, 1932.

Symmetrical enlargement of the gums, involving either localized areas of the upper or lower jaw or more diffusely the entire jaw margin, has been described under three designations: hypertrophic gingivitis, symmetrical fibroma and symmetrical osteofibroma. In the first two forms, which shade off into each other, only the soft tissues are involved. In the third form, which is not to be sharply delimited from the second, there is now formation of bone from the alveolar processes. From a review of the literature and the study of five cases that he presents, Koblin concludes that the process is essentially inflammatory and not blastomatous. It may begin as an acute productive gingivitis due to inflammation set up by trauma or foreign bodies, or it may begin as infection and inflammation of the peridental membrane and alveolar processes. It is a progressive proliferative process, and what have been described as three conditions are simply stages. For these stages Koblin prefers the names chronic hyperplastic gingivitis, fibrositis and osteofibrositis. The condition begins most often in the second or third decade of life. Etiologic factors are: heredity; age, but only in so far as it relates to the eruption of the permanent teeth; inflammation, which is the most frequent cause and may be due to infection, foreign material or faulty eruption of teeth; endocrine dysfunction; pregnancy, and disturbances of the central nervous system.

O. T. SCHULTZ.

Microbiology and Parasitology

ACTINOMYCOSIS OF THE HYPOPHYSIS CEREBRI AND ACTINOMYCOTIC LEPTOMENINGITIS. M. N. ZAJEWLOSCHIN, Frankfurt. Ztschr. f. Path. **43**:335, 1932.

This is a case of a 24 year old man with the diagnosis of diabetes insipidus and syphilitic meningo-encephalitis. About three weeks before admission the patient became blind. At autopsy the diagnosis was apical tuberculosis of the right lung, tuberculous leptomeningitis and caseous tuberculosis of the hypophysis. Histologically, however, neither the hypophysis nor the meninges revealed any evidence of tuberculosis. Instead, a typical actinomycosis of the hypophysis and actinomycotic leptomeningitis were found. The author was able to find only one similar case in the literature.

O. SAPHIR.

TWO CASES OF HUMAN PSEUDOTUBERCULOSIS ASSOCIATED WITH HEMOCHROMATOSIS. W. UMLAUFT, Virchows Arch. f. path. Anat. **280**:18, 1931.

The author reports two cases of human pseudotuberculosis, bringing the total number of reported cases of this disease to seven. The clinical duration was nine days and ten days respectively, and the clinical course was that of typhoid. From each case there was cultivated an organism held to be the same as Pfeiffer's bacillus of rodent pseudotuberculosis. Postmortem examination revealed the presence of hemochromatosis in each case. The author believes that the hemochromatosis was an independent and primary condition, which, however, had an effect on the course of the pseudotuberculous infection because of the widespread involvement of the reticulo-endothelial system.

O. T. SCHULTZ.

BACTERIOLOGY OF LYMPHOGRANULOMATOSIS AND MYCOSIS FUNGOIDES. NATALIA BUSNY, Virchows Arch. f. path. Anat. **280**:626, 1931.

In one hundred and forty cases of lymphogranulomatosis and five of mycosis fungoides, the author cultivated from the blood and involved tissues a bacterial organism that is morphologically and culturally identical in the two diseases. The ordinary bacteriologic mediums and methods were used. Within from twelve to twenty-four hours the organism appears in cultures as a slender, acid-fast bacillus. In from twelve to twenty-four hours more it is transformed into gram-positive cocci that are arranged in pairs, tetrads and short chains. In an additional twelve to twenty-four hours the cocci have increased in size and have arranged themselves in staphylococcus-like clusters. Injection of the acid-fast bacillary forms into guinea-pigs leads to the formation of lesions like those in the human diseases. From such lesions the pleomorphic organism can again be cultivated. For the successful inoculation of guinea-pigs a larger amount of inoculum is necessary than in the case of the tubercle bacillus. If the coccoid forms are injected into guinea-pigs, only the acid-fast bacillary form is obtained from the animal after forty-eight hours. The localization of the organism in the experimental lesions is identical in both lymphogranulomatosis and mycosis fungoides. The author considers the organisms specific for lymphogranulomatosis and mycosis fungoides. Further immunobiologic investigation is necessary to determine whether the organisms from the two diseases are identical and to determine their relationship to the tubercle bacillus.

O. T. SCHULTZ.

SEARCH FOR STREPTOCOCCI IN THE EARLY LESIONS OF ACUTE RHEUMATIC INFECTION. F. KLINGE and C. MCEWEN, Virchows Arch. f. path. Anat. **283**:425, 1932.

This is the fifth in the series of articles devoted by Klinge and his co-workers to acute rheumatic infection. The object of the present study was the demonstration of streptococci in the lesions. Previous findings have been based on the examination of rather older lesions of the proliferative stage. Klinge's material included two patients who died on the seventeenth and nineteenth days of acute rheumatic infection, the lesions being of the early fibrinoid-degenerative type described in previous communications. Material from various sources from these two cases, as well as that from older lesions in other cases, was used for study. In a large number of sections carefully examined no streptococci could be found. The negative results do not deny the etiologic significance of the streptococcus, especially if the allergic theory of rheumatic infection is accepted, since experimental investigation has shown that hyperergic tissue changes may be produced in sensitized animals by such a small number of streptococci that the latter cannot be found in the lesions.

O. T. SCHULTZ.

THE INFLUENCE OF RYE AND OF WHEAT BREAD ON RATS AND MICE. E. FRIEDBERGER and H. NARJES, Ztschr. f. Immunitätsforsch. u. exper. Therap. **74**:447, 1932.

Of 83 rats and 220 mice, those fed exclusively with rye bread lived much longer and exhibited greater resistance to spontaneous infections (pneumonia and enteritis) than did the animals fed with wheat bread. The latter group gained more weight than the former.

I. DAVIDSOHN.

THE BIOLOGIC VARIABILITY OF DYSENTERY BACILLI. I. E. RUTSCHKO, Ztschr. f. Immunitätsforsch. u. exper. Therap. **74**:468, 1932.

From 393 strains of dysentery bacilli, 23 biochemical types and 4 serologic groups were isolated. When further observed they showed a tendency to change into other biochemical types. On numerous occasions more than 1 serologic type

and various transitional forms were isolated from the same patient. The frequency of such findings increased with the number of the examined colonies, which suggests that the usual technic of examining from 1 to 3 colonies of a fecal spread cannot give the full information. From the finding of multiple types, usually only during the end-stages of the disease, in chronic cases and in carriers, it is concluded that the variants develop within the body of the host.

I. DAVIDSOHN.

THE VARIABILITY OF DYSENTERY BACILLI AND THEIR ETIOLOGIC IDENTITY. I. RUTSCHKO, Ztschr. f. Immunitätsforsch. u. exper. Therap. **74**:500, 1932.

The various types of dysentery bacilli are "mutations" or "permanent modifications" of one common type. That conclusion was arrived at after a study of 415 strains, belonging to the various types. Some of the types, such as B. E-Kruse-Sonne, Flexner and Y, gave variants about 12 times more frequently than, for instance, B. Shiga-Kruse. In no case was there observed a transition of any of the types into B. Shiga-Kruse. The rôle of the bacteriophage was definitely established as an etiologic factor in the mechanism of mutations.

I. DAVIDSOHN.

THE MECHANISM OF INFECTION PARTICULARLY IN MICE WITH BACILLUS ABORTUS. TAGE HELMS, P. HOLM and J. ORSKOV, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:55, 1932.

In guinea-pigs given injections of B. abortus (Bang) a systemic infection developed, with characteristic hemorrhagic, edematous, local reactions at the site of injection. Mice were successfully infected parenterally and orally (the latter mode of infection in mice being the first on record). The number of organs from which the bacteria could be recovered was greater in the parenterally infected mice, and the infection lasted longer in that group. In mice infected orally, the bacteria were first found in the submaxillary and mesenteric lymph nodes, and only after varying periods of time did a more or less generalized infection result. In neither type of infection was a bacteremia noted, at any rate, not as a manifestation of a primary invasion of the blood.

I. DAVIDSOHN.

THE DISTRIBUTION OF SPIROCHETES OF TYPE WEIL IN FREIBURG-I.-BR. AND VICINITY. ERICH KUNSTEIN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:173, 1932.

The nonpathogenic Spirochaeta pseudo-icterogenes was found in about 60 per cent of forty-eight samples of water obtained from various sources. The temperature of the water is not an important factor, as the organism was found even at 0 C., but it seems to thrive better in a slightly alkaline medium. The pathogenic Spirochaeta icterogenes was not found in the water of that vicinity, but was present in eight of nineteen examined rats. Intraperitoneal injection of pulp, prepared from the kidneys of the animal carriers, into guinea-pigs gave more positive results than direct dark-field examination. Older rats were more frequently found to be infected.

I. DAVIDSOHN.

EXPERIMENTAL INFECTION OF MICE WITH TYPHOID BACILLI. WALTHER MENK and WALTER SCHREIBER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:503, 1932.

The white mouse is recommended for chemotherapeutic studies of typhoid infections. Typhoid bacilli were successfully inoculated by the subcutaneous, intravenous and intraperitoneal routes, and in two cases by direct injections into liver and kidney. The success of the inoculation was determined by postmortem bac-

teriologic studies of the organs at various intervals after the injections. In some cases the bacilli were also found in the urine and feces obtained during the life of the animals. In animals examined during the first seven weeks after inoculation, typhoid bacilli were recovered in a large majority of cases. In only three of sixteen mice which were fed with typhoid bacilli did infection take place.

I. DAVIDSOHN.

Immunology

SEROLOGICAL REACTIONS WITH SIMPLE CHEMICAL COMPOUNDS (PRECIPITIN REACTIONS). K. LANDSTEINER and J. VAN DER SCHEER, J. Exper. Med. **56**:399, 1932.

Experiments are described demonstrating the precipitation of azodyes by immune serums prepared by the injection of azoproteins containing the same azo-component. These precipitin reactions prove conclusively the view already advanced on the basis of inhibition reactions, that antibodies combine specifically with substances of small molecular weight. Although in this respect both phenomena have the same significance, the precipitin reactions with dyes are simpler in that the aid of a protein antigen is eliminated. That specific serologic precipitin reactions can take place with substances other than proteins has been amply demonstrated by studies on bacterial antigens (polysaccharides). The present findings show that for precipitation with immune serums not even a high molecular weight of the reactive substance is required. Factors determining the tendency to separate from the liquid on combination with antibody seem to be the colloidal state of the solution and the chemical composition of the substance. With regard to the influence of chemical composition, a striking example is provided by the suberic acid dye, which gives particularly strong precipitin reactions, most probably because of its long aliphatic side chains. The results reported may be of use for studies on the mechanism of serologic precipitation.

AUTHORS' SUMMARY.

RÔLE OF LEUKOCYTOLYSINES OF THE SPLEEN IN ANAPHYLACTIC SHOCK. G. E. SSACHAROFF and O. W. KRASSOWSKAJA, Virchows Arch. f. path. Anat. **284**: 312, 1932.

The normal leukocytolytic activity of the spleen is appreciably reduced in the early period following the injection of a sensitizing foreign protein. This function of the spleen may be stimulated by mild roentgen irradiation of the spleen or by small doses of antispleen serum, and may be depressed by larger doses of antispleen serum. In a variety of experiments on dogs designed to alter the leukocytolytic activity of the spleen, the effect of the leukocytolysines on anaphylactic shock was studied. Although in some instances the reduction of the leukocytolytic action of the spleen by means of antispleen serum or spleen extract decreased the severity of the shock, the results of the experiments were so variable that no definite conclusions could be drawn.

O. T. SCHULTZ.

SIGNIFICANCE OF THROMBOZYTOBARINE AS A DEFENSIVE MECHANISM IN INFECTION. I. L. KRITSCHEWSKI and A. M. BRUSSIN, Zentralbl. f. Bakt. (Abt. 1) **120**:150, 1931.

Mice infected with Trypanosoma equiperdum were treated with arsphenamine. When a heavy suspension of trypanosomes was injected intravenously into these animals, the blood platelets deposited themselves on the trypanosomes almost immediately, and the phenomenon could be seen in the heart's blood, lungs, liver and spleen. The injection of immune serum into heavily infected animals gave the same effect. Similar results were obtained with Spirillum duttoni.

The authors concluded that the thrombozytobarine acts on the specific antigen and changes its surface so that colloidal aggregations occur, thus decreasing the

degree of dispersion. The colloidal aggregates are then removed from the circulating blood, especially in the liver, spleen and lungs, where they are either lysed or engulfed and destroyed by phagocytes.

PAUL R. CANNON.

BACILLARY IMMUNITY IN DIPHTHERIA. E. HOEN and L. TSCHERTKOW, Zentralbl. f. Bakt. (Abt. 1) **120**:320, 1931.

The authors gave guinea-pigs subcutaneous injections of cultures of diphtheria bacilli (Park no. 8) which had been washed to remove traces of toxin and killed by 0.5 per cent phenol. Eighteen days later these and normal animals were tested intracutaneously with diphtheria toxin; they reacted alike, which indicated the absence of antitoxin in the vaccinated animals. Similarly, injections with living bacilli killed the vaccinated at the same rate as the normal animals. The authors, conclude, therefore, that immunity to diphtheria is antitoxic rather than antibacterial.

PAUL R. CANNON.

DO TUBERCULOUS AND NONTUBERCULOUS SERUMS POSSESS BACTERICIDAL PROPERTIES FOR THE TUBERCULOSIS BACILLUS? O. KIRCHNER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **74**:56, 1932.

Undiluted serum was unsatisfactory for the growth of tuberculosis bacilli, but improved markedly when properly diluted. There was no evidence of specific bactericidal properties for the tuberculosis bacillus in thirty-two tuberculous and twenty nontuberculous serums. The differences which were noted could be explained on the basis of physicochemical and nutritive factors.

I. DAVIDSOHN.

TUBERCULIN SENSITIVENESS FOLLOWING INFECTION WITH TUBERCULOSIS BACILLI OF VARYING VIRULENCE. P. UHLENHUTH and W. SEIFFERT, Ztschr. f. Immunitätsforsch. u. exper. Therap. **74**:101, 1932.

The skin sensitiveness to tuberculin in tuberculous guinea-pigs depends on the virulence of the strain which produced the infection. This holds true also for the avirulent B C G bacillus, which sensitized similarly as a human and as a bovine strain of low virulence. Guinea-pigs infected with a B C G strain succumbed to only rather high doses of tuberculin injected intraperitoneally, while in other strains of varying virulence the amounts required for a fatal intraperitoneal injection did not show any relation to the virulence of the infecting strain, as it was seen with regard to skin sensitiveness. Tuberculin sensitiveness is not a measure of immunity but an indicator of the pathogenicity of the bacilli. Cutaneous and general desensitization resulted from intraperitoneal injections of sublethal doses of tuberculin.

I. DAVIDSOHN.

VARIATIONS IN RESISTANCE TO INFECTIONS WITH STREPTOCOCCI FOLLOWING IMMUNIZATION WITH HETEROBACTERIA. S. OTSUKI, Ztschr. f. Immunitätsforsch. u. exper. Therap. **74**:249, 1932.

Preliminary injections of killed B. coli and of B. proteus raised the resistance of rabbits but did not fully protect them against fatal doses of hemolytic streptococci. A mixture of the two bacteria was still more effective. B. pestis and B. typhosus lowered the resistance. Various other bacteria had no noticeable effect one way or the other.

I. DAVIDSOHN.

INFLUENCING ANAPHYLACTIC SYMPTOMS AND SHOCK WITH ORGAN EXTRACTS. B. PAUL and E. RÓTH, Ztschr. f. Immunitätsforsch. u. exper. Therap. **74**:270, 1932.

Anaphylactic shock was prevented in sensitized guinea-pigs by intraperitoneal or enteral administration of protein-free splenic and liver extracts. Extracts of

stomach and muscles had only feeble protecting effect. In patients with eosinophilia the intramuscular administration of a splenic extract tended to lower the number of eosinophilic leukocytes.

I. DAVIDSOHN.

ACQUIRED IMMUNITY IN PLANTS INDUCED BY GRAFTING. DONTCHO KOSTOFF, Ztschr. f. Immunitätsforsch. u. exper. Therap. **74**:339, 1932.

The development of precipitins, lysins and proteolytic reacting substances was studied in certain Solanaceae, following the production of interspecific and intergeneric graft combinations with proper controls. The ring method, the nephelometric estimation and the colorimetric dialyzation-ninhydrin reaction were employed. The reagents were extracts and dialyzates of leaves and stems. The development of immune precipitins and of proteolytic substances was established.

I. DAVIDSOHN.

REMOVAL OF ANAPHYLACTOGENIC PROPERTIES OF SERUM. W. KULIKOFF, A. BEILINSSON and W. TSCHERNOCHWOSTOFF, Ztschr. f. Immunitätsforsch. u. exper. Therap. **74**:389, 1932.

The effect of certain anticoagulants on the removal of anaphylactogenic properties of serum is due to two factors: (*a*) their stabilizing effect and (*b*) the denaturing of the protein substances brought about by changes in the p_H during the acidification and subsequent alkalinization. These properties are much more sensitive to the procedure than the antitoxic properties, as evidenced by almost quantitative preservation of the antitoxic units in an antidiphtheritic serum. It is possible that the effect on the anaphylactogenic properties of serums of some older concentration methods, particularly those employing acid solutions in the procedure, was due more to the change of the p_H than to the partial removal of the protein substances.

I. DAVIDSOHN.

SEROLOGIC STUDIES IN NONSYPHILITIC PATIENTS INOCULATED WITH TERTIAN MALARIA. KARL ELLER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **74**:397, 1932.

The literature dealing with the behavior of the Wassermann reaction in patients with malaria, but free from syphilis is reviewed. While the reports on this subject vary a great deal, there is hardly any doubt that false positive Wassermann reactions occur frequently in nonsyphilitic patients inoculated with malaria. The presence of fever, the interval since inoculation, the quinine therapy and various other factors are of importance for the presence or absence of the positive complement-fixation test. The author studied 168 nonsyphilitic patients whose gonorrheal infection was treated with malaria and 22 seronegative syphilitic patients who were similarly treated. The Wassermann reaction, with various antigens (also with a syphilitic liver extract), the Sachs-Georgi precipitation reaction and the Meinicke *Truebung* reaction were employed. Four or more attacks of fever preceded the appearance of a positive test. The frequency of the positive reactions depended on the test employed, being most frequent with the complement-fixation test, less frequent with the precipitation test and rarely positive with the Müller reaction. The last-mentioned test is the most sensitive of the three in syphilis. The evaluation of various factors suggests that the positive reactions in syphilis and in malaria are based on different, though superficially similar, changes.

I. DAVIDSOHN.

THE LYSOZYME AND FLOCCULATION PHENOMENON OF CHICKEN EGG-WHITE. E. FRIEDBERGER and F. HODER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **74**:429, 1932.

Only one of eighty-three saprophytes isolated from the air was markedly sensitive to the lysozyme of egg-white. The lysozyme could not be absorbed

by treatment with sensitive bacteria, but was removed by adsorbing substances like animal charcoal, bolus alba and kaolin. It could not be separated from the adsorbing substances. Some of the bacterial strains, both those which were dissolved by the lysozyme and many of those which were not dissolved by it, were flocculated by dilutions of chicken egg-white. The flocculation was distinctly different from agglutination. Boiling destroyed the lysing and flocculating ability of the egg-white and of the bacteria. High concentrations of sodium chloride also had an inhibiting effect on both phenomena. It is probable that the lysing and flocculating phenomena are due to the same factor. While the lysozyme did not affect pathogenic bacteria, some of the bacteria were flocculated by the egg-white.

I. DAVIDSOHN.

FUNCTION OF THE SPLEEN IN BARTONELLA INFECTION OF THE WHITE RAT. HANS ROTH, Ztschr. f. Immunitätsforsch. u. exper. Therap. **74**:483, 1932.

A few days following splenectomy the latent Bartonella muris-ratti appeared in the erythrocytes in large numbers, reaching the maximum around the sixth day, and then slowly disappeared from the circulating blood. About 80 per cent of the animals died within fourteen days. The surviving animals had repeated recurrences. Occasional micro-organisms were seen in the erythrocytes of nonsplenectomized animals. The mortality was considerably decreased, and the duration of life was prolonged by feeding the splenectomized animals with liver. After partial splenectomy the resistance of the rats was unaffected as long as at least one fifth of the organ remained. High voltage roentgen therapy directed toward the spleen and exhaustion of the animals by running did not decrease their resistance to the Bartonella.

I. DAVIDSOHN.

MECHANISM AND PROPHYLAXIS OF THE ANAPHYLACTIC SHOCK. E. ZIMMERMANN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:1, 1932.

Potassium was found to be increased in the blood of rabbits and guinea-pigs during anaphylactic shock and also following injections of various substances, producing on parenteral injection anaphylactoid symptoms, such as histamine and peptone. In the rabbit the potassium level was considerably higher during a true anaphylactic shock than during an anaphylactoid reaction, while in the guinea-pig such a difference was not observed. The accumulation of potassium was associated with a delayed resorption from the blood, as compared with normal animals. Pronounced symptoms appeared in rabbits when the potassium reached a level of from 50 to 60 mg. per hundred cubic centimeters; the lethal level was at 65 mg. and higher. In guinea-pigs both levels were lower. Injection of potassium chloride was followed in guinea-pigs by development of typical pulmonary emphysema, having the appearance of that seen in anaphylactic shock. Injections of various substances having a nonspecific desensitizing effect (substances producing a blockade of the reticulo-endothelial system and homologous serum, atropine and magnesium) increased the ability of the animals to eliminate potassium from their blood, so that much larger quantities of potassium chloride had to be injected to reach the level at which symptoms appeared.

I. DAVIDSOHN.

VACCINATION OF MICE AGAINST BACILLUS ABORTUS. TAGE HELMS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:61, 1932.

Injection of small doses of killed bacilli modified the course of the subsequent infection with living bacilli administered parenterally or orally. In vaccinated animals, bacteria were recovered from only the regional lymph nodes. Very large subcutaneous injections protected some animals against fatal intraperitoneal injections.

I. DAVIDSOHN.

EXTRACT OF SUPRARENALS OF THE HORSE AS ANTIGEN FOR THE WASSERMANN REACTION. GERD STERN-STRÄTER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:71, 1932.

This extract proved satisfactory in about 35,000 tests, together with syphilitic liver extract and with cholesterolized beef heart extract. False positive reactions were rare (such a possibility had to be considered in only ten cases).

I. DAVIDSOHN.

PHYSICOCHEMICAL QUALITIES OF THE ISOHEMO-AGGLUTINOGENS. VERA SCHROEDER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:77, 1932.

The group-specific lipoids, obtained by extracting erythrocytes with alcohol and subsequent evaporation of the extract, were able to bind the corresponding agglutinins. They are thermostable (resist boiling for twenty-four hours) and are not destroyed by ultraviolet rays. They are adsorbed easily and later given up by kaolin and aluminum hydroxide U. S. P., the fixation by the latter being somewhat more stable. The adsorption with coal is easily accomplished in the case of group B, while group A is not adsorbed by it. The iso-electric point of the group-specific lipoids is around p_H 2.7, which indicates that they belong to the group of lecithins. Chemical analysis showed the absence of protein substances and the presence of a mixture of various saturated and nonsaturated fatty acids. When the lipoid-agglutinogens react with the corresponding euglobulin-agglutinins, a protein-lipoid complex results, which differs in its physicochemical qualities (its surface tension and iso-electric point) from the constituent substances in their pure condition.

I. DAVIDSOHN.

PRODUCTION OF THE "BALL" PHENOMENON IN COLLOIDAL SOLUTIONS OF CHOLESTEROL AND IN CHOLESTEROLIZED LIPOID MIXTURES BY HYDROCHLORIC ACID AND SODIUM CHLORIDE. ALFRED MODEL, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:100, 1932.

The "ball" reaction of Müller can be produced by addition of high concentrations of sodium chloride and of hydrochloric acid to cholesterolized beef heart extracts, even in the absence of syphilitic serum, thus showing that it is a colloid phenomenon. That phenomenon is produced easily in the presence of syphilitic serum, but it can also be brought about by the presence of electrolytes. The cholesterol is the essential component, as is best demonstrated by the possibility of producing the phenomenon when using cholesterol alone without the tissue extracts. Variations in the concentration of sodium chloride did not affect the reaction with syphilitic serums. The quantitative relations between the tissue extracts and the added cholesterol, the technical variations in the preparation of the mixtures, the length of maturation and the temperatures employed for the reaction exerted a profound influence on the result, which showed itself in the rapidity of the formation of the ball, in its shape and in its stability. By proper variation of the experimental conditions, a ball was formed within only fifteen minutes.

I. DAVIDSOHN.

CHEMICAL NATURE OF THE SO-CALLED SYPHILIS ANTIGENS. TIBOR RÓNAI, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:125, 1932.

Rabbits treated with a mixture of the cholesterol-free beef heart extract of Kiss plus pig serum produced an immune serum which fixed complement with the homologous antigen and with a cholesterolized beef heart extract, while rabbits given injections of tissue extracts previously treated with adsorbing substances, which removed their ability to react with syphilitic serums, did not produce such immune serums. The rabbit immune serums reacted with the adsorbed tissue extracts, but alcohol precipitation of the rabbit serums according to Sachs and Georgi removed their ability to react with such modified antigens, leaving the reaction with native

antigens intact. The Wassermann reaction, artificially produced in the rabbit by immunization with proper tissue extracts, is identical with the Wassermann reaction occurring in syphilis. The spontaneous positive Wassermann reaction in the rabbit and the nonspecific positive Wassermann reaction in man occurring in certain diseases are due to the presence in the serum of unstable globulin substances, which are able, in the presence of tissue extracts, to inactivate complement and to produce precipitates.

I. DAVIDSOHN.

EFFECT OF LIGHT ON THE BACTERICIDAL PROPERTIES OF SERUM. ALFRED PETTERSSON, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:156, 1932.

The ultraviolet rays destroyed the bactericidal properties of serum much more effectively than did the visible rays. The complement was considerably more sensitive than the bacteriolysines and the bactericidal products of leukocytes.

I. DAVIDSOHN.

Tumors

TUMOR OF THE PINEAL BODY. H. STRAUB, Frankfurt. Ztschr. f. Path. **42**:250, 1931.

One case is described. A man, 19 years old, with the clinical diagnosis of intracranial tumor, died four days after ventricular puncture. The anatomic diagnosis was: lobulated tumor of the region of the pineal body; compression of the sylvian aqueduct and fourth ventricle; marked internal hydrocephalus involving the lateral and third ventricles; compression of the pons and cerebellum. The tumor measured from 40 to 35 mm.; it was well circumscribed and nodular. It was in the region of the pineal body. No evidence of this structure was found. The tumor consisted of cells closely resembling the ones found in the normal pineal body. They were arranged in the form of alveoli, which were surrounded by connective tissue fibers. The cells showed a small amount of cytoplasm and well defined nuclei, which contained several well stained granules. There were areas of hemorrhage throughout the tumor. There was no evidence of malignancy. Even though no structures absolutely characteristic of the pineal body were found, Straub believes that the tumor took its origin from that body because of its location, because it was so well defined, and because the cell resembled those found in the normal pineal body.

O. SAPHIR.

UNUSUAL SITUATION OF TUMORS OF THE TYPE OF MIXED TUMORS OF THE SALIVARY GLANDS. W. KREIBIG, Frankfurt. Ztschr. f. Path. **42**:281, 1931.

A man, 38 years old, had a tumor on the right leg in the region of the tibia. It was the size of an apple, situated beneath the skin, and easily movable. The clinical diagnosis was fibroma. The tumor was removed without difficulty, and the periosteum and tibia were examined at the operation but no changes were noted. The growth was firm, lobular and grayish white, and had a shiny cut surface. Histologically, the tumor consisted in part of connective tissue with only a few nuclei. The connective tissue fibers separated nodular areas, consisting of glandular structures and a peculiar granular material which in some portions stained blue, in others red, if stained with hematoxylin-eosin. There also were many spindle-shaped cells with spindle-shaped nuclei and a cytoplasm which showed distinct ramification. The glandular structures were lined by two layers of cells, partly high cylindric and partly low cuboid. Their lumens were either empty or contained an eosinophilic material. Areas consisting of squamous cells were found, with typical horn pearls. The various possibilities of the origin of this tumor are discussed. Also, another case is mentioned which possibly falls into the same group.

O. SAPHIR.

STRUCTURE OF THE MALIGNANT TUMOR CELL. B. LIPSCHÜTZ, Virchows Arch. f. path. Anat. **282**:107, 1932.

In this article, Lipschütz summarizes work carried on over a number of years, the detailed results of which have been published from time to time in other journals. The aim of the work has been the discovery of morphologic characteristics that distinguish the cells of malignant tumors from other cells. By the use of a modified wet Giemsa stain method, he claims to have discovered such characteristics. A specific feature of the malignant tumor cell is that he terms the "plastin reaction" of its cytoplasm. This term is used because the staining reaction is like that of plastin or nucleolar substance, although any relation of the cytoplasmic material giving the plastin reaction to nucleolar substance or to any nuclear derivative is denied. The reaction is evidence of a diseased state of the cell, and that diseased state manifests itself in malignant proliferation. The reaction has not been obtained in some tumors. No diagnostic value is claimed for the reaction, which appears to be influenced by the hydrogen ion concentration of the tissue. The material that gives the specific plastin reaction is transformed into or replaced by another specifically characteristic material that occurs in the form of numerous small, unstained bodies to which he gives the name stegosomes. These have been detected regularly in the Rous chicken sarcoma and in transplantable sarcoma and chondrosarcoma of the mouse, but only infrequently or not at all in other animal tumors or human neoplasms, although a footnote claims more recent uniform success in smear preparations of such tumors. Hypertrophy of the archoplasm, nuclear alterations, and abnormalities of the chromosomes, previously described by many writers, are not characteristic of tumor cells and are not limited to the latter. They are the result, not the cause, of the abnormal state of the cell that expresses itself biologically as malignancy and morphologically as the plastin reaction. A supplementary section describes the criteria for distinguishing between plasma cells and tumor cells.

O. T. SCHULTZ.

CANCER MORTALITY IN CHARKOW (U. S. S. R.). A. M. MERKOW and E. I. PALTSCHEWSKY, Virchows Arch. f. path. Anat. **282**:158, 1931.

Although extensive statistics of cancer from the countries of western Europe are available, those from the Ukraine are fragmentary. In an analysis of 647 cases of cancer in almost 10,000 autopsies in Charkow from 1901 to 1929 the important facts derived are: 1. Mortality from cancer is not increasing. 2. Clinical diagnosis is improving, although more cases are being diagnosed cancer which are not substantiated at autopsy. 3. The average age at death is 51 years. This is ten years younger than in the countries of western Europe. 4. There is no increase in frequency of cancer in the younger age groups. 5. The ratio between males and females is 2:1. 6. By far the most frequent site of occurrence is the stomach. 7. Metastasis occurred in 57 per cent of the cases.

PERRY J. MELNICK.

RHABDOMYOMA. G. L. DERMANN and Z. W. GOLBERT, Virchows Arch. f. path. Anat. **282**:172, 1931.

The authors describe the histologic aspects of a partially differentiated rhabdomyoma of the tongue and one of the vocal cord. In a brief supplement, a second similar tumor of the tongue is described.

O. T. SCHULTZ.

GENERALIZED FIBROUS OSTEITIS ASSOCIATED WITH PARATHYROID TUMOR. D. BERNER, Virchows Arch. f. path. Anat. **282**:680, 1931.

In two typical cases of generalized fibrous cystic osteitis, a single enlarged parathyroid body was found at necropsy. Both patients were women. One was aged 48 years. The other, whose age is not stated, had had 8 children, the youngest 7 months old. In the first case, the tumor was 18 mm. in diameter. In the second, it was the size of the end of the thumb. Histologic examination led

Berner to conclude that both were benign adenomas. In his discussion of the relation of parathyroid tumor and hyperparathyroidism to generalized fibrous osteitis, he tabulates twenty-four operative cases. In sixteen, an enlarged parathyroid body was found at operation, and in 8 an enlarged body was not found. The failure to find a parathyroid tumor may be due to the abnormal situation that the parathyroid bodies may have or to hyperfunction without demonstrable enlargement. The association of an enlarged parathyroid body with osteomalacia he explains on the ground that the hyperplastic parathyroid body may not be in an actively hyperfunctioning state.

O. T. SCHULTZ.

SALIVARY GLAND TUMORS COMPOSED OF ONCOCYTES. H. HAMPERL, Virchows Arch. f. path. Anat. **282**:724, 1931.

In the salivary glands and in the mucous glands of the tongue, mouth, pharynx, esophagus and air passages there occurs a type of cell that was first described by Schaffer in 1897 and that was later named pyknocyte by Zimmermann because of the deeply stained, irregularly contoured nucleus. The cytoplasm of these cells is granular and meshed, and the cell appears swollen. The nucleus is situated nearer the free border than the base of the cell. Hamperl considers the cytoplasmic characteristics more important than those of the nucleus. Such cells, which he calls oncocytes or tumor cells, are derived from both acinar and duct epithelium. Systematic examination showed them to be absent from the salivary glands up to the age of 20 years and present in increasing numbers with increasing age above 20 years. Hamperl describes two small, nonencapsulated, tumor-like masses of such cells encountered in his examination of salivary glands. He describes further an encapsulated tumor situated near the opening of a salivary duct. It was composed of a relatively large amount of lymphoid tissue and of cylindric epithelium which had a papillary arrangement in some places and a glandular arrangement in others. Histologically, the tumor was identical with that described by Albrecht and Arzt in 1910 as a papillary cystadenoma of lymphoid tissue and later termed adenolymphoma. This type of tumor, which some have derived from misplaced salivary gland tissue and others from persistent branchial cleft tissue, is apparently identical with that which American writers have described as lympho-epithelioma. Hamperl believes that the epithelial cells of such tumors are derived from or are identical with oncocytes. Oncocytes he believes to be epithelial cells in a state of dedifferentiation or senile differentiation.

O. T. SCHULTZ.

PSEUDOMYXOMA OF PERITONEUM OF APPENDICAL ORIGIN. W. DIEKER, Virchows Arch. f. path. Anat. **282**:761, 1931.

Peritoneal pseudomyxoma originating in mucocele of the appendix has led to discussion of the question whether all the mucoid material is formed in the mucocele, from which it escapes by rupture of the latter, or is locally formed in the peritoneal cavity from epithelium misplaced from the mucocele. The case reported is presented as evidence of the secretion of the mucoid material by misplaced epithelium. In a large, sausage-shaped, cystic mass continuous with a mucocele of the appendix, and in smaller cysts of the pelvis and lower part of the abdomen, all of which were filled with thick mucoid material, there were found areas with cylindric, mucus-secreting cells like those of the mucosa of the appendix.

O. T. SCHULTZ.

HISTOLOGY OF GLIOMA. E. F. REYNOLDS and J. K. SLATER, Virchows Arch. f. path. Anat. **282**:772, 1931.

In their study of fifteen gliomas, Reynolds and Slater paid especial attention to the cellular structure and to the character of the peripheral portions. In thirteen, the clinical symptoms indicated active growth, whereas in two the symptoms pointed to quiescence. In twelve of the thirteen actively growing gliomas, the peripheral portion consisted of syncytium; in the other, it was composed of syncytium in

which were young astrocytes. Three of the tumors were composed wholly of syncytium; one of these had been clinically quiescent for four years. Many of the tumors exhibited all stages of differentiation from syncytium through a spongioblast stage to well differentiated astrocytes. The authors are not certain that the latter are capable of division. The growth of most of the tumors was infiltrative; in a few it was expansive. In every case there was active increase in the number of the astrocytes of the surrounding original tissue. Necrosis was noted not only in the older parts of the tumors, but also in the younger, syncytial portions. Hemorrhage of varying degree was frequent; in some cases, it occurred at a considerable distance from the tumor. The authors voice a brief objection to the modern classification of the gliomas, so far as the pigeon-holing of a given tumor under a particular name may lead to neglect of the study of the biologic properties and characteristics of the tumor.

O. T. SCHULTZ.

HISTOLOGY OF MALIGNANT EMBRYONIC ENCHONDROMA. K. MAKRYCOSTAS, Virchows Arch. f. path. Anat. **282**:787, 1931.

An enchondroma of the upper end of the femur occurred in a woman aged 79. The cartilage that formed the essential tissue was embryonic and consisted of small cells of variable shape embedded in a cartilaginous matrix in part hyaline and in part fibrous. The interstitial, expansive growth of the tumor led to compression atrophy of the surrounding bone. The outer thin shell of bone that covered the tumor was periosteal in origin. Areas of differentiated cartilage were formed, as were also lamellae of bone. The mucoid and cystic degeneration of the tumor was vascular in origin. In the degeneration, the lamellae of the original spongy bone, which were included within the tumor, were freed, and in some instances again became surrounded by bone of enchondral origin. The destruction and reformation of bone was repeated a number of times.

O. T. SCHULTZ.

MYELOMA IN A CHILD. K. ZÄH, Virchows Arch. f. path. Anat. **283**:310, 1932.

Zäh claims that this is the third reported case of myeloma in childhood. The tumor involved the fourth and fifth cervical vertebrae and caused compression of the spinal cord. No other bones were involved, but the lung contained a number of small metastases. The neoplasm was composed of myeloblasts and contained groups of erythroblasts.

O. T. SCHULTZ.

LOCALIZATION AND METASTASIS OF PRIMARY CARCINOMA OF PANCREAS. F. WALLAU, Virchows Arch. f. path. Anat. **283**:321, 1932.

In four and one-half pages Wallau presents an interesting statistical summary of 330 cases of primary carcinoma of the pancreas. His figures are not based on cases recorded in the literature, but on the protocols of necropsies in 104 cases from various institutions with which G. B. Gruber has been connected and in 226 collected by a commission of German pathologists shortly after the World War. In age incidence, the seventh decade led with 31.2 per cent; the sixth decade was next with 22.7 per cent; the fifth and eighth decades followed in the order named with 18.8 and 13.9 per cent. There were two cases in the second decade. Fifty-nine and three-tenths per cent were in males and 40.7 per cent in females. The head of the pancreas was the primary site of localization in 49.8 per cent, the tail in 8.5 per cent, the body in 4.8 per cent; in 36.9 per cent, the primary site could not be determined from the protocols. Multiple primary localization was noted 9 times: 5 times in the head and body, 3 times in the head and tail, and 1 in body and tail. Metastasis occurred in 82 per cent of the cases, and was 10 per cent less frequent in carcinoma of the head than of the body or tail. The slightly lower incidence

of metastasis of carcinoma of the head is ascribed, not to a lower degree of malignancy, but to earlier occlusion of the common duct by carcinoma of the head. Metastasis occurred much more frequently to the liver and regional lymph nodes than to any other situation. The peritoneum, lung, pleura and kidney were the next most frequent sites of metastasis in the order named. O. T. SCHULTZ.

Medicolegal Pathology

AUTOMOBILE RUPTURE OF THE INTESTINES. H. HAMILTON COOKE, Ann. Surg. **96**:321, 1932.

Twelve cases of traumatic rupture of the intestines were found in 210 victims of automobile accidents who came to necropsy at the department of pathology, University of Minnesota, between Dec. 1, 1919, and Dec. 1, 1929. The site of the rupture was as follows: duodenum, 3 cases; jejunum, 6 cases; ileum, 2 cases, and ascending colon, 1 case. In 3 cases the rupture of the intestines was associated with rupture in the stomach. In 3 cases the rupture was transverse and in 1 case nearly transverse, while in 8 cases the rupture varied from 5 to 15 mm. in diameter. The liver, spleen, kidneys or pancreas were injured in 5 cases. In 7 cases there were no abrasions of the skin or any other external evidences of injury to the abdomen. In 4 cases there were a few superficial abrasions, while severe lacerations of the wall of the abdomen were found in only 1 case. Free blood in the peritoneal cavity was present in 11 cases. Peritonitis was present and was the principal cause of death in 4 cases. In 6 cases (50 per cent), death occurred within one hour after the injury was received. In this group the rupture of the intestines was associated with rupture of the spleen, liver, pancreas, kidneys, fracture of the skull, subdural hemorrhage, multiple fracture of the ribs and hemothorax. In the other 6 cases, the rupture of the intestines was the most serious injury and with its complications was the cause of death. The symptoms and signs were identical with those present in traumatic rupture of the intestines from causes other than automobile accidents. The duration of life was from seven to forty-eight hours after the accidents occurred. Death was the result of peritonitis in 5 cases, and of peritonitis and bronchopneumonia in 1 case. Rupture of the intestines should be considered as a possibility, whenever a person has received a severe blow on the abdomen in an automobile accident. Lacerations and wounds of the wall of the abdomen are infrequent, and their absence is not a reliable criterion of the condition of the intestines, viscera or mesentery.

FROM AUTHOR'S SUMMARY.

POISONING FROM DRINKING RADIUM WATER. A. O. GETTLER and C. NORRIS, J. A. M. A. **100**:400, 1933.

This paper presents the first case on record of fatal poisoning from the consumption of water containing traces of radium salts, in which the presence of radium in the bones and tissues was definitely proved by scientific laboratory procedures. Death was due to pathologic lesions brought about by the radium, which entered the system by water which was drunk for a few years, and which was said to contain 2 micrograms of radium in every 2 ounces (60 cc.) of water. The presence of the radium was proved by both the electroscopic and the photographic method. The quantity of radium in the bones and soft tissues was determined by the emanation method. The entire skeleton contained 73.27 micrograms of radium; the soft tissues, only 0.39 micrograms. The total amount of radium, therefore, in the entire body was 73.66 micrograms. Radium salts (or radioactive substances in general), when introduced into the blood stream, become detrimental to health and ultimately fatal, and therefore the use of foods or beverages containing radium salts should be prohibited.

AUTHORS' SUMMARY.

DILUTION OF CHLORIDES IN BODY FLUIDS AS A SIGN OF DROWNING IN FRESH WATER. J. LECLERC, M. MULLER and J. PAYEN, Ann. de méd. lég. **8**:528, 1932.

Experiments were made by submerging guinea-pigs in distilled water. The concentration of chlorides in the body fluids was diminished after the submersion. When the results are applied to man, the sources of error are postmortem degeneration, drowning in salt water and rapid death at the moment of falling into the water. The authors have studied fifteen cases of human drowning in thirteen of which there was an exact history. Dilution of the chlorides is not a pathognomonic sign of death from drowning. Dilution does, however, help to establish the diagnosis of death from drowning. The method needs further study.

H. S. THATCHER.

FRACTURES OF THE LARYNX BY DIRECT VIOLENCE. PIÉDLIÈVRE, DESOILLE and ROUSSEL, Ann. de méd. lég. **8**:562, 1932.

Fractures of the larynx due to hanging, strangulation by cord and with the hand have been studied systematically, but those caused by direct trauma have not been carefully studied. A case is reported of a man struck on the neck by a blow of the fist; grave symptoms of asphyxia developed, and he died several hours afterward. At autopsy edema of the glottis and a bloody infiltration about the larynx were found, also the following fractures: (1) a median and vertical fracture of the thyroid cartilage; (2) a fracture of the left posterior portion of the cricoid cartilage, and (3) a fracture of the right great horn of the hyoid bone. A review of the literature revealed that fracture of the cricoid cartilage is relatively infrequent, while fracture of the thyroid cartilage is relatively frequent. Fractures of the arytenoid cartilages, hyoid bone and some of the first rings of the trachea are exceptional. The mechanisms of the fractures are reviewed. Age and ossification increase the liability to fracture. Death occurs rapidly. Fractures of the larynx from direct violence should not be overlooked in efforts to find the cause of death.

H. S. THATCHER.

PHOSPHORUS POISONING. J. SEDLMEYER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **19**:365, 1932.

A description of three fatal cases of phosphorus poisoning is given. In the first case, a 25 year old woman took phosphorus to induce an abortion; the second case, which is also of criminalistic significance, involved a man, aged 57, poisoned by his wife; in the third instance, an uxoricide is described. In each case the results of the autopsy and the chemical examinations are presented in detail.

E. L. MILOSLAVICH.

COOPERATION OF PHYSICIAN AND CHEMIST IN DETECTION OF FATAL POISONING. H. MERKEL, Deutsche Ztschr. f. d. ges. gerichtl. Med. **19**:384, 1932.

Merkel emphasizes the importance of scientific team-work between the experts in legal medicine and in forensic chemistry. Often a microscopic or bacteriologic examination of the organs discloses the real cause of death and eliminates an erroneously assumed poisoning. Acute yellow atrophy of the liver may closely resemble, clinically, acute phosphorus poisoning. Paratyphoid infections may occasionally deceive one into assuming an ingestion of arsenic. In protracted cases of phosphorus poisoning, the chemical examination, as a rule, fails to reveal elementary phosphorus.

E. L. MILOSLAVICH.

Technical

A Tissue Substrate Microculture for Tubercle Bacilli. H. J. Corper, J. A. M. A. **99**:1315, 1932.

This method is based on the fact that from 1 to 2 volumes of 6 per cent sulphuric acid will destroy undesirable contaminators and saprophytic acid-fast bacilli within from one-half to one hour at 37 C., and that blood or egg yolk, after the treatment with sulphuric acid, neutralized with an isotonic solution (1.3 per cent) of sodium bicarbonate containing 3 per cent glycerin, is a good nutrient medium for supporting the growth of small numbers of human tubercle bacilli at 37 C. In control laboratory tests and in routine empiric tests with sputums negative on microscopic examination of smears, the simple tissue substrate microculture has revealed a high percentage positive for tubercle bacilli, which recommends the test for this purpose in practice.

Author's Summary.

Constant Temperature Water Baths. F. W. Bishop, C. M. Carpenter and S. L. Warren, J. Exper. Med. **56**:719, 1932.

A series of six water baths in one unit, set at 37, 39, 40, 41, 41.5 and 42 C., for the purpose of studying the thermal death time of bacteria and of body tissues has been described.

Authors' Summary.

The Estimation of the Number of Organisms in Suspensions of Tubercle Bacilli. O. Kirchner and B. D. Li, Beitr. z. Klin. d. Tuberk. **80**:454, 1932.

The number of living bacilli in suspensions of tubercle bacilli is best determined by the method of Lange (*Ztschr. f. Tuberk.* **46**:455, 1926). In this method the different concentrations are seeded on egg medium, and the number of colonies are counted.

Max Pinner.

The Conditions for the Isolation of Tubercle Bacilli from the Blood. O. Kirchner and B. C. Li, Beitr. z. Klin. d. Tuberk. **80**:460, 1932.

Neither serum nor plasma of tuberculous or nontuberculous patients has any bactericidal action, nor has the whole blood. If blood is directly inoculated, a minimum of 100 bacilli must be present in 1 cc. in order to produce growth. It is preferable to inoculate serum mediums according to the author's method (*Zentralbl. f. Bakt.* (Abt. 1) **124**:403, 1932). In this case a one hundred times greater volume of blood can be used, and, as demonstrated previously, tubercle bacilli grow well in the depth of serum mediums. Tubercle bacilli are present in much larger numbers in the leukocytic layer of centrifugated blood than in other parts of the blood. The most successful culture method is, then, to inoculate the upper cellular layer of centrifugated blood into serum mediums.

Max Pinner.

The Diastase Content of the Cerebrospinal Fluid in Syphilis. A. Marcionini and B. Ottenstein, Klin. Wchnschr. **28**:1424, 1932.

The authors showed previously that the diastase content in the cerebrospinal fluid of nonsyphilitic persons ranged from 11 to 40 and in some instances to 80 mg. per hundred cubic centimeters, while in fluids of syphilitic persons the diastase content ranged from 0 to 10 mg. The disappearance of the diastase was ascribed to the deleterious effect which the poisons of Spirochaeta pallida have on the diastase-forming function of the central nervous system. The studies now reported include further results and conclusions as follows: 1. Low diastase content (from 0 to 10 Gm.) was found in 100 per cent of untreated patients with clinical manifestations of neurosyphilis (cerebral syphilis, tabes dorsalis, progressive paralysis), and in 80.2 per cent of patients similarly afflicted, but treated. 2. Low spinal fluid

diastase was demonstrated in 79 per cent of untreated persons with syphilis who were free from clinical manifestations of central nervous system involvement (secondary syphilis, tertiary syphilis, latent syphilis and congenital syphilis), and in 60.3 per cent of persons similarly afflicted, but untreated. 3. The Wassermann reaction of the blood and the occurrence of low cerebrospinal fluid diastase do not run concurrently in all cases. 4. Testing the diastase content of cerebrospinal fluids is offered as a laboratory adjunct in the diagnosis of syphilis. 5. Compared with the Wassermann reaction, cell count, and the colloidal gold reaction, the diastase test appears to be more sensitive.

B. S. LEVINE.

THE CULTIVATION OF TUBERCLE BACILLI FROM BLOOD BY LÖWENSTEIN'S METHOD. D. DIMTZA and H. GUTSCHER, München. med. Wchnschr. **79**:1917, 1932.

With Löwenstein's technic the authors could not demonstrate bacteremia in tuberculous patients. In 112 blood cultures from 70 patients (40 had bone tuberculosis), only 1 positive result was obtained and that was in a case of miliary tuberculosis. Acid-fast rods of unknown nature and detectable only microscopically were found in the blood of a patient with tuberculosis of the knee.

D. O. ROSBASH.

STAINING OF INTRACELLULAR FAT IN TISSUE CULTURES. Z. SZANTROCH, Virchows Arch. f. path. Anat. **286**:675, 1932.

The author describes a combined fixing and staining method for demonstrating fat in tissue cultures. He uses a mixture of 4 parts of saturated sudan III in 95 or 70 per cent alcohol and 1 part of solution of formaldehyde. He also describes a simple method for the vital staining of fat in tissue cultures with sudan III.

O. T. SCHULTZ.

STAINING OF INSOLUBLE CALCIUM SALTS IN TISSUES. G. GÖMÖRI, Virchows Arch. f. path. Anat. **286**:682, 1932.

The usual methods for the microchemical demonstration of calcium in the tissues are unsatisfactory because they must be applied after decalfication of the tissue. The author presents a method in which silver impregnation of the calcium salts precedes decalcification. The preparations are permanent and withstand the action of light.

O. T. SCHULTZ.

Society Transactions

NEW YORK PATHOLOGICAL SOCIETY AND NEW YORK ACADEMY OF MEDICINE, SECTION OF SURGERY

Joint Meeting, Nov. 4, 1932

PAUL KLEMPERER, *President, Presiding*

TUMORS OF THE THYROID GLAND: PATHOLOGIC ASPECTS. ALLEN GRAHAM, Cleveland.

Benign Tumors.—Only adenomas will be considered. From the standpoint of etiology, antecedent goiter is more important in the development of adenoma than heredity and infection. Simple endemic goiter seems to be a more potent antecedent than hyperthyroidism.

For purposes of discussion, it may be assumed that goiter, in its broadest sense, is an overgrowth of thyroid tissue, not of inflammatory origin and not neoplastic. It is encountered in two phases, an actively growing or hyperplastic phase and an involuting or resting phase.

The immediate cause of the overgrowth of the thyroid is an inadequate storage of iodine in the gland itself. The storage of iodine in the gland may be affected by an inadequate intake of iodine, faulty assimilative functions and increased requirements for iodine. Correction of these factors results in the accumulation of iodine within normal limits; it stops or lessens the intensity of the hyperplastic reaction, and initiates involution. Complete involution constitutes what is called colloid goiter and is comparable to complete involution of the breast after lactation.

Viewed from this standpoint, the hyperplastic reaction in the thyroid may be considered specific for the depletion of stored iodine, but it certainly is not specific for any particular clinical syndrome.

The applicability of this conception lies in two directions:

1. It explains the impossibility of accurately correlating the clinical disorders with the histologic changes in the thyroid.

2. Depletion of stored iodine, however brought about, is responsible for the overgrowth of the thyroid which constitutes goiter, and it is from the goitrous thyroid that adenoma arises.

Two theories as to the mode of origin of adenoma are worthy of consideration. Wölfler attributed its development to the growth of embryonal rests; this theory was widely accepted for many years. Serious objection was raised by those who unsuccessfully searched for the embryonal rests in the thyroids of embryos, fetuses and the newly born. The theory failed to account for the occurrence of typical so-called fetal adenomas in the thyroids of adults; the histologic characteristics of these nodules indicated recent new growth rather than long-standing neoformations.

More recently what may be termed the hyperplasia-involution theory appears to be supplanting Wölfler's conception. According to this theory, the adenomas arise directly from the epithelium of the thyroid, and the transformation may be initiated at any age. The theory is acceptable because it seems to be in accord with experience.

After the adenomatous neoformation has been initiated, the nodule may grow for a time and then remain quiescent indefinitely; it may have alternate periods of growth and quiescence; it may undergo degenerative changes, or it may become malignant. It presents the greatest variety of histologic pictures, from the highly undifferentiated fetal type of tissue to the fully differentiated adult thyroid vesicles filled with colloid. It has the capacity of storing iodine and of elaborating active thyroid secretion.

Recently there has been considerable discussion as to whether or not so-called lobular hyperplasia (nodular goiter) and adenoma are two separate pathologic conditions. I am thoroughly convinced that so-called lobular hyperplasia and adenoma are distinct departures from the normal structure, that with the increasing departure from the normal they approach the status of definite neoplasms and that a fundamental distinction between them is unwarranted on the basis of pathogenesis or pathologic anatomy.

It is certain that only a relatively small percentage of patients with adenomatous goiter feel the need of consulting a physician. An adenoma becomes of clinical importance when its size and position cause symptoms of a mechanical nature, when it becomes inflamed or infected, when it is associated with symptoms of hyperthyroidism or when it becomes malignant.

Malignant Tumors.—The etiologic importance of endemic goiter in the development of adenoma has been emphasized. The etiologic importance of adenoma in the development of carcinoma is equally striking.

Malignant Tumors of the Thyroid, 1905-1929; Results of Follow-Up Study as of October, 1932

	Number of Patients	Dead	Alive	Not Traced
No operation	48	31	5	12
Operation (no sections available)	15	9	1	5
Parastruma	1	1	0	0
Epidermoid carcinoma (primary)	1	1	0	0
Sarcoma:				
Myxosarcoma	1	1	0	0
Lymphosarcoma	11	8	2	1
Spindle cell	5	3	1	1
Sarcocarcinoma	5	4	1	0
Carcinoma:				
Unclassified	16	13	2	1
Scirrhous	6	6	0	0
Papillary	15	6	9	0
Malignant adenoma	78	46	25	7
Carcinomatoid (?) (adenocarcinoma, not in adenoma)	17	0	16	1
Multiple types	3	0	3	0
	222	129	65	28
Adenoma—discard	108	4	82	22
	330			

The most frequently encountered malignant tumor is carcinoma, which constitutes approximately from 80 to 85 per cent of all malignant epithelial growths in the thyroid. Probably more than 90 per cent of these malignant epithelial growths have their origin in preexisting benign adenomas. It is the transformation from benign to malignant adenoma which constitutes the pathologic entity in the vast majority of instances.

The circumstances of the transition afford a satisfactory explanation of the incalculable histologic variations in the malignant growth. All stages of growth, differentiation, dedifferentiation, regression, degeneration, metaplasia, proliferation, invasion and dissemination, may be encountered in a series of tumors, and many of them in a single tumor.

It is necessary for the pathologist to adopt rather precise standards by which to judge the benignancy or the malignancy of an adenoma. Such standards, it is believed, are now available. They have been gradually evolving for a number of years. They are founded on an extensive experience with the pathologic changes in the thyroid, and have been checked by a critical analysis of the end-results of treatment.

The experience at the Cleveland Clinic is summarized in the accompanying table, which includes all of the cases observed from 1905 to 1929, inclusive. The results of the follow-up study are as of October, 1932.

The cases fall readily into the groups indicated in the table, with rather inconsequential overlapping. The individual groups have rather well defined characteristics which make classification not difficult.

Particular attention is directed to the group designated "Adenoma-Discard." All of these, at some time in the past, have been considered by me and by others as representing varying degrees of malignancy, based almost entirely on their histologic characteristics. In none of these has there been recurrence or metastasis after surgical removal. The group has been followed for an average of more than six years, in many instances for ten years and in some cases for from fifteen to seventeen years.

MALIGNANT TUMORS OF THE THYROID GLAND. GUILFORD S. DUDLEY.

One feature of the malignant tumors of the thyroid is the variability of their microscopic pictures. Not infrequently the determination of a final diagnosis is dependent on close cooperation between the pathologist and the clinician. Statistics appear to be in accord that malignant tumors exist or arise in association with less than 2 per cent of nodular goiters.

The outstanding preoperative diagnostic criterion of a malignant process is hardness to palpation, plus the rather rapid and progressive enlargement of an existing tumor. In the cases of cancer in which the patients were cured permanently by operation, the diagnosis was not made clinically, but was established histologically after operation. The presence of a symptomless tumor in the thyroid is undesirable. It has been observed that such tumors are more prone to cause symptoms of hyperthyroidism than to become malignant.

In the absence of any contraindication, the decision in favor of operative removal serves to rid the patient of the menace of symptoms of hyperthyroidism, as well as the possible but improbable menace of a malignant disease. Although metastases occur in a large proportion of cases of cancer of the thyroid, these deposits are of slow growth, and usually death is due to local causes.

DISCUSSION

DAVID MARINE: Dr. Graham's discussion of tumors of the thyroid was a very conservative one, with which I heartily agree. Dr. Graham has, I believe, accurately referred to the mode of origin of the so-called adenomas. They arise in some such fashion as Dr. Graham has indicated, due to hyperplasia and involution, succeeding each other over many years. Succeeding hyperplasias and involutions disturb the uniformity of the supply of blood. The groups of thyroid cells that are in the best relation to the supply of blood will grow out first. I think that the theory that the variation in the supply of blood and the cycles of hyperplasia and involution follow each other over a long period is the best explanation available at present.

In regard to the question of malignant epithelial tumors of the thyroid, I again agree with the conservative grouping of Dr. Graham.

There are some general features of tumors of the thyroid which have always aroused my interest to which Dr. Graham did not refer. The first one of these dates back to Cohnheim, who described cases of benign metastasizing adenoma. Morphologically, the thyroid tissue in the metastases looks like good physiologically active thyroid. It contains iodine and thyroxine. The patient may live from ten to twenty years after the metastases are noted. Is such a tumor a malignant tumor, a carcinoma? Since the thyroid tissue is very hardy an autogenous transplantation can be made with the greatest ease, even after the tissue has been kept in the icebox for eight days. I believe that in the presence of adenomas that are superficial and exposed to trauma, rupture of the sinusoid spaces can easily occur, and a bit of the thyroid can be carried into the blood stream to some other part of the body, where it acts as an ordinary autograft.

Morphologic criteria of a malignant condition of the thyroid are difficult to find, and, as Dr. Graham pointed out, one has to look for invasion of the capsule and the blood vessels as the most reliable criterion.

One other feature that has interested me is the question of the function of tumors of the thyroid. Dr. Graham referred to the fact that with respect to simple adenomas there is no question that they do function. Do the malignant tumors function? Many of them undoubtedly do, but the association of exophthalmic goiter with cancer of the thyroid is comparatively rare, as compared with the association of exophthalmic goiter with adenoma. The association is not as common as it is with tuberculosis, syphilis and other diseases. In an audience of surgeons every one immediately thinks of von Eiselsberg's case in which he removed a carcinoma of the thyroid and produced myxedema; with the recurrence of the tumor, the myxedema cleared up. I think that his observation may have been incomplete. He may not have removed all of the nontumorous thyroid tissue, and if he did not, it may have regenerated. It has been my good fortune to work with a number of carcinomas of the thyroid in the dog. The relation of carcinoma of the thyroid to antecedent goiter is the same in the dog or in brook trout as it is in man. The incidence of carcinoma of the thyroid is greatly increased in districts where goiter is common. I have given iodine over varying periods to dogs with carcinoma of the thyroid, and in those with metastases I have not yet found an instance in which there was any evidence of the storage of iodine. There is some evidence that these malignant tumors of the thyroid in man and in dogs are not capable of functioning. I do not think that the rare instances in which exophthalmic goiter is associated with malignant tumors offer an evidence of function of the tumor. A great deal of good work could be done in further studying the relation of the metabolism of iodine to malignant tumors. There must be all grades of tumors, as there are all grades of malignancy.

LAWRENCE W. SMITH: Dr. Graham spoke of struma lymphomatosa, a condition in which I am particularly interested. I am not yet convinced that these small round cell tumors are actually of lymphoid origin. I have seen eleven cases, most of which are conclusively epithelial; in a few, notably three, there is less certainty as to the nature. One case has been registered with the Lymphatic Tumor Registry, and thus far no agreement has been reached; three men favor carcinoma, and five, struma lymphomatosa. As I recall it, the relationship between struma lymphomatosa (Hashimoto's disease), Riedel's struma and small round cell tumors, whether lymphosarcomas or carcinomas, is perhaps only of academic interest, but may be of some clinical significance. So far I have seen only one case of this type in which the condition responded temporarily to irradiation. One is inclined to be guided by this clinical test as much as by the morphology, which I am willing to admit is a most inconclusive procedure. The radioresistance is rather definitely in favor of the epithelial rather than the lymphoid origin of these tumors.

The same thing is true to a less definite extent in cases of so-called fibrosarcoma. I have seen two cases in which no definite evidence of an epithelial origin could be traced, but in most of the cases that have been regarded as cases of fibrosarcoma, I think one could always trace a definite origin from the epithelium, with spindling-out of the cells, as Dr. Ewing described. Both of these groups occur relatively late in life; they are seldom seen in patients under 50. They run a rapid course; the patients usually have a history of preexisting adenoma, with a sudden increase in the size of the tumor over a period of only a few weeks or months, and the patients are almost invariably dead within from eight months to a year. There have been two cases in which the patients have lived as long as from two to three years, but this is such a small number that the cases are of relatively little significance.

RUSSEL PATTERSON: The special point that I should like to discuss is the possibility of earlier diagnosis in these cases. The points concerning diagnosis brought out by Dr. Dudley are really the signs that develop after the malignancy has progressed, and hardly any of them will give the slightest suggestion of early disease. All hyperplastic processes in the thyroid gland vary because (1) the thyroid has an unusual capacity for independent growth; (2) the structure of the gland is such that its vascularity is enormous and variable, and its colloid secretion varies rapidly in quantity and composition, and (3) the gland responds remarkably to functional stimuli, such as drugs, diet and the action of other glands in the body.

A malignant tumor may develop in any patient's goiter. The tremendous number of possibilities makes it almost impossible to say whether a malignant tumor may develop, or whether it may be present in the early stages. The situation is discouraging, and as it now stands, when one can diagnose clinically a malignant condition of the thyroid, it is too late.

J. WILLIAM HINTON: How should one treat patients with a nodular or an adenomatous type of growth? Some investigators have recommended removing all growths in the early stages. That certainly is not necessary, because some of the nodular growths are not true adenomas, but are involutional types of enlargement, which will clear up under medical treatment. The percentage is small, but I have seen just as many tumors clear up as became malignant. Two per cent, if treated properly medically, may clear up. Dr. Dudley's statistics show that actually only 2 per cent of his clinical tumors turned into carcinomas, so the question is this: Should one advise a patient with a nodular goiter to have it out as a prophylactic measure? One can be misled by the hardness of the tumor, because we all know that calcification is present in nodular adenomatous types of tumors, which becomes clear on roentgenograms, but there are also carcinoma and calcification in some cases. The length of the disease apparently has to be considered as a factor. If a patient has had a nodular goiter for a number of years which is of a hard consistency, it probably should be removed.

The other problem is: What should one do with the patients after they have been operated on? In my experience, of fifteen cases of carcinoma, eleven were of the malignant adenomatous type. None of these patients (followed for from six months to seven years) has died, and none of them received roentgen therapy after operation. Whether it was a mere coincidence that they received roentgen therapy before carcinoma developed, or whether the therapy had any bearing on the development of the carcinoma, I do not know. I should like to ask Dr. Graham whether he advises roentgen therapy for the malignant adenomatous type of tumor. Of course, the scirrhous type is always fatal.

ARTHUR S. MCQUILLAN: These interesting papers bring out the fact that the relationship of benign to malignant tumors of the thyroid tissue is more confusing than the relationship of such tumors in many other types of tissue.

Dr. Graham stated that the majority of adenomas of the thyroid are secondary to, or result from, endemic goiter. This appears contrary, at least, to my experience. I have seen nodules or so-called adenomas of the thyroid develop suddenly in persons who have been perfectly normal before so far as disturbances of the thyroid are concerned. Such instances associated with the strain of childbirth, severe coughing, singing and the blowing of musical instruments are common enough. It is reasonable to assume that hemorrhage results, leading to the growth of a nodule. In fact, gross hemorrhage into the thyroid tissue is seen frequently, followed by an adenoma after the clinical signs of hemorrhage have disappeared.

Has it been noted that exophthalmic goiter or even toxic adenoma is associated in a greater or lesser degree with carcinoma of the thyroid, especially the diffuse rapidly growing carcinoma? This has been my experience in a few cases.

Would it be possible for the intense but long-standing hyperplasia of exophthalmic goiter to grow into a carcinoma? I have seen two or three cases the histories and course of which pointed in this direction.

In regard to Dr. Dudley's discussion concerning the percentage of carcinoma in his group of patients with tumors of the thyroid operated on, I may say that our figures agree.

There is one tumor, a rare tumor of the thyroid gland, which no one mentioned. This is the Hürthle cell tumor that Dr. Ewing described. It apparently originates from parathyroid tissue that has been misplaced in the thyroid gland. I have seen one case.

I believe that every adenoma should be treated surgically. Dr. Dudley would lead us to believe that he is somewhat more conservative in this respect.

FREDERIC W. BANCROFT: Dr. Graham stated that a number of cases have been removed from the presumably malignant group and put in the adenomatous

class. I should like to ask him whether he did that on a review of the pathologic material entirely, or was it on the basis of the life history? Also has he had to put any of the benign adenomas back into the malignant group on account of the sudden death of the patient?

JAMES EWING: With Dr. Smith in recent years I have been interested and much puzzled by the difficulty in diagnosis between struma lymphomatosa and small cell carcinoma. In the beginning, when encountering cases of struma, I was inclined to regard all of the tumors as comparatively benign Hashimoto tumors, with the possibility of lymphosarcomatous changes in some of them, but in recent years I have come to the conclusion that there is not infrequently a small cell carcinoma of the thyroid which it is extremely difficult to distinguish from struma lymphomatosa.

I am still unconvinced that there is any pathologic significance to sarcoma of the thyroid. In a case of Dr. Meeker's, I was inclined to think that the tumor was a neurosarcoma and not a sarcoma of the thyroid. I do not believe that a primary sarcoma of the thyroid exists in the sense in which it is generally assumed.

Concerning the metastasizing strumas of the thyroid, years ago I was thrilled with the idea that a benign tumor can metastasize, running a course which is malignant, and yet retain a benign histologic aspect. Looking back over many cases, I feel that all of them show histologic evidence of carcinoma, and since the clinical course of all of these cases, so far as I know, has been that of carcinoma, I am inclined to think that the so-called benign metastasizing struma of the thyroid is a carcinoma.

Dr. Marine has referred to a study of the physiology of tumors of the thyroid. Recently, Plaut demonstrated the thyroid nature of certain ovarian tumors. It would be well for pathologists to employ this test to determine whether different forms of tumors of the thyroid are capable of functioning.

Finally, all of the reports give a pessimistic outlook for the patient with a carcinoma of the thyroid which is recognizable clinically. I wonder, then, whether it would be worth while to consider such a revolutionary step as recommending irradiation in all cases in which clinical experience has shown that carcinoma is liable to develop. From the evidence of the clinician it is practically impossible to recognize many malignant tumors at a time when one can do the patient any good. We may therefore be forced to assume that it is wise to employ irradiation in a routine way in those cases in which we know carcinoma is liable to develop. I merely leave this suggestion for the clinicians, who have the responsibility of accepting or rejecting it.

DR. ALLEN GRAHAM: It may have been misleading to present a case of struma lymphomatosa along with the lymphosarcomas. This was done in order to call attention to what I believe may be a source of lymphosarcoma in a few cases. Experience is too limited to appreciate fully the possible relationship of these two lesions. I would emphasize that the eleven cases in the table were not related in any way to struma lymphomatosa.

I believe that a number of the cases reported by Dr. Smith under the title, "Small Round-Cell Carcinomata," are in reality cases of lymphosarcoma.

Dr. Ewing raised objections to the group of cases called spindle cell sarcomas. I am aware that in some cases of carcinoma of the thyroid the tumor cells may assume the spindle shape and in other ways simulate sarcoma, and I have rigorously excluded this type of case from the list of sarcomas.

Dr. Ewing raised the question as to the advisability of irradiation of adenoma and adenomatous goiter as a prophylaxis against carcinoma. This question may be prompted by a misconception of the natural hazard of cancer confronting a patient who has an adenomatous goiter. While it is true that about 90 per cent of carcinomas develop from adenomas, only an incalculably small percentage of patients with adenomatous goiter ever have carcinoma. Of the surgically removed thyroids, about from 1 to 2 per cent are malignant; this figure is not infrequently cited as indicative of the danger of cancer, which is a great exaggeration of the natural incidence.

With reference to the group of adenomas that have been discarded from the list of malignant tumors, I have followed these cases for varying periods up to seventeen years, and in not one instance has there been a recurrence or a metastasis, and no patients have died from malignant disease of the thyroid. In no case has there been invasion of the capsule or of the blood vessels, a criterion that is of greater importance in prognosis than are the histologic aspects of the tumors.

I have never seen a tumor consisting of parathyroid tissue situated in the thyroid. I have seen parathyroid-like tissue in an adenoma of the thyroid.

In from about 30 to 40 per cent of cases of malignant goiter symptoms somewhat suggestive of hyperthyroidism may be present. I have never seen a frank case of hyperthyroidism associated with a true malignant tumor, although such cases have been reported in the literature.

There seems to be little probability of improving clinical recognition of a malignant process in an early stage. In no organ or tissue of the body is the prevailing type of malignant growth so dependent on the preexistence of a benign tumor. There is no possibility of recognizing the early stages of the transition. The prognosis is dependent on the type of growth and the extent of involvement. The prognosis is best for patients with carcinomatoid tumors, which are practically benign; it is next most favorable in cases of papillary cystadenocarcinoma; the malignant adenomas, in which the tissue is predominantly adenomatous, are next in the order of increasing malignancy. Very few patients with malignant adenomas predominantly carcinomatous survive for three years, and even fewer survive five years; the end-results in cases of all other types of malignant goiter are disappointing.

Dr. Marine referred to Cohnheim's original case of "metastasizing benign struma." In all instances in which the thyroid has been examined, adenomatous nodules have been present. There need be no mystery about the establishment of secondary deposits from adenomatous nodules (but not from normal thyroid tissue) through the blood stream. Irrespective of whether or not these nodules are considered carcinomas or simple implantations, the majority of patients have died of the disorder.

Dr. Marine also referred to von Eiselsberg's famous case. The symptoms regressed or disappeared with the simultaneous development of a secondary growth in the manubrium. Dr. Marine suggested that the disappearance of the myxedema might have been due to regeneration of unremoved thyroid tissue, and not to the assumption of function by the metastatic nodule. Perhaps he has overlooked the fact that later the manubrial tumor was removed, and the symptoms of myxedema recurred. I still think that von Eiselsberg's case offers a certain amount of evidence indicating the possibility of functional capacity in certain tumors.

Dr. Guilford S. Dudley: Concerning the difficulty of diagnosis of early malignant adenomas, we are apparently not able at present to surmount that difficulty.

PATHOLOGICAL SOCIETY OF EASTERN NEW YORK

Arthur W. Wright, *Secretary*

Regular Meeting, Dec. 9, 1932

Victor C. Jacobsen, *Presiding*

Granulosa Cell Tumor of the Ovary. R. J. Lebowich.

A married white woman, aged 55, was admitted to the hospital with the clinical history characteristic of granulosa cell tumor of the ovary as defined by TeLinde, namely, regular menstruation starting in after the menopause.

At operation, the pelvic cavity was filled with about 1,200 cc. of dark fluid blood. The right ovary was converted into a large ovoid mass, surrounded by a smooth, dense capsule which was ruptured at its upper pole, and measured 20 by 12 by 10 cm. The upper two thirds of the tumor was occupied by blood clot marbled

with dirty yellowish-brown necrotic tissue. The remainder of the mass was honeycombed by multiple smooth thin-walled cysts measuring up to 1 cm. in their widest diameter, filled with pink fluid material or blood and surrounded by firm, smooth, grayish tumor tissue.

The tumor was composed of crowded follicle-like agglomerations or cylinders of tumor cells; the cells were polyhedral, stained uniformly and presented very few mitoses in preparations fixed in a fluid made up as follows: solution of formaldehyde, U. S. P., 500 cc.; distilled water, 1,500 cc.; glacial acetic acid, 100 cc., and trinitrophenol to the point of saturation. The cysts were lined by either elongated theca-like tumor cells or granulosa-like tumor cells, and often contained degenerated tumor cells. There were frequent minute areas varying from vacuolation of tumor cells to beginning liquefaction and cyst formation. The stroma was hyaline, and was well vascularized by capillaries. There were no primordial ova. The capsule was not invaded.

LYMPHOBLASTOMA OF THE SPLEEN WITH SPLENECTOMY. VICTOR C. JACOBSEN.

CASE 1.—A Jew, aged 54, a tailor, had complained of weakness and a dragging pain in the left side for seven months. Eight months before death, he had a severe, sharp abdominal pain on the left side, with a temperature of 104 F. The following three months he was free from pain, but lost 30 pounds (13.6 Kg.). On admission to the hospital, he complained of weakness, night sweats and frequent vomiting unrelated to food or to pain.

The spleen was palpable below the level of the umbilicus. There was marked ascites. The icteric index was 16.5. The blood picture showed secondary anemia with a normal leukocyte count: large mononuclears, 3.7 per cent; lymphocytes, 20.5 per cent.

Splenectomy was performed by Dr. A. W. Elting on Nov. 21, 1932. Following this the leukocytes rose to 37,850, 90 per cent being polymorphonuclears. The hemoglobin was 73 per cent; the red blood cells numbered 3,900,000. Death occurred one month later; leukemia did not develop.

The spleen measured 24 by 16 by 9 cm., and weighed approximately 1,500 Gm. The capsule was tightly stretched, purplish blue and for the most part smooth and glistening, but showed some areas with delicate fibrous adhesions. Areas of infarction were noted bulging beneath the capsule.

On section, a large infarct was revealed, about 9 cm. in diameter, with smaller infarcts grouped around it. The splenic substance was dark red, and the edges tended to evert, although the tissue was moderately firm. Many minute thrombi were widely distributed.

The enlargement of the spleen appeared to be due primarily to hypertrophy of the lymphoid follicles, which were from 1 to 2 mm. in diameter. In the sinuses were megakaryocytes, red cells and an increased number of polymorphonuclear cells. The germinal centers were large and of varying contour. The predominating cell was the large lymphocyte, although small lymphocytes were abundant. Hemosiderin was abundant about the vessels in the lymphoid nodules.

The diagnosis was lymphoblastoma of the spleen.

At autopsy, acute peritonitis and acute mesenteric lymphadenitis were present. There was no generalized lymphadenopathy, but one large node in the mesentery suggested early Hodgkin's disease more than simple lymphoblastoma.

CASE 2.—A white man, aged 43, consulted Dr. A. W. Elting because of a cutaneous lesion on the face, a basal cell carcinoma. Enlargement of the spleen and severe secondary anemia were present. There was no enlargement of the liver. A clinical diagnosis of Banti's disease was made, and a splenectomy was performed four weeks later by Dr. Elting.

The spleen weighed 2,730 Gm., and measured 25.5 by 20.5 by 8.5 cm. The color was normal, and there were a few adhesions in the neighborhood of the hilus. There was an accessory spleen, weighing 30 Gm. and measuring 5 by 3.8 by 3 cm. The consistence of the large spleen was slightly increased. Blood drained from the splenic vein; it clotted with normal rapidity and was of normal color. In the

midportion of the lower anterior mesial surface was a groove 7 by 2.3 cm. The cut surface disclosed rather uniform hypertrophy of the lymphoid follicles, which attained a maximum diameter of 3 mm. The follicles were pale, white and semi-translucent. The pulp in between was correspondingly scant and pale red. The veins were large and distended; the trabeculae were inconspicuous. The appearance was that of splenomegaly due to hypertrophy of the lymphoid tissue. There was no significant fibrotic change. The accessory spleen showed similar changes. There were about six lymph nodes in the region of the hilus, the largest being 3 by 2 by 1.5 cm., and the smallest, 0.5 cm. The nodes were discrete and definitely enlarged, but paler than the spleen, although they were redder than the normal lymph nodes.

On microscopic examination, the white nodules diffusely scattered through the spleen proved to be much enlarged malpighian bodies. The lymphogonia or lymphoblasts were not very conspicuous, the predominating cell being of small lymphocytic type. Mitotic figures were present, but not in an excessive proportion. The pulp was correspondingly decreased in amount, and the blood in the sinuses showed a few nucleated erythrocytes but no excess of pigment. There were a few eosinophils in almost every high power field, with an occasional megakaryocyte. The veins of the spleen were rich in lymphocytes. The capsule was essentially normal. As was to be expected, the accessory spleen presented the same picture as the main spleen.

The enlarged lymph nodes from the splenic hilus also had greatly hypertrophied germinal centers, small and large lymphocytes predominating. The lymphoblasts were of less than normal prominence. In the peripheral lymph sinuses, in addition to tremendous numbers of lymphocytes, phagocytes stuffed with erythrocytes and some with blood pigment were present. Nucleated erythrocytes were seen in moderate numbers. The central sinuses were distended with lymphocytes. There was no apparent invasion of the capsule, although some capillaries in the capsule were surrounded by lymphocytes.

The blood picture at no time showed a tendency toward leukemia. Following the operation the leukocyte count rose to 28,000, but gradually dropped to normal six months later. The hemoglobin varied between 65 and 75 per cent (Sahli). The monocytes were 10 per cent at the last count, showing a definite increase, but further observations will be necessary to determine whether this drift is going to be continued. Eight and a half months after splenectomy, the patient had gained weight, his color had improved, and his only complaints were "gas on the stomach" and indigestion. No lymph glands were palpable.

SOLITARY PLASMA CELL MYELOMA. ARTHUR W. WRIGHT.

An American, aged 62, married, was admitted to the hospital complaining of a "lump on the breast bone." This lump had been present for about three months, beginning as a small elevation but growing quite rapidly until it was about the size of half an orange. The family history was not important.

The patient was well nourished and stocky; he was sitting quietly in bed without any pain or discomfort. The weight was 217 pounds (98.4 Kg.); the height, 71 inches (182.9 cm.). The temperature, pulse rate and respiratory rate were normal. The blood pressure was 145 systolic and 70 diastolic. Nothing of note was found except for the tumor of the chest. This was located in the midline over the upper third of the sternum. It projected outward as a smooth, hard circular mass about 10 cm. in diameter at the base and 4 cm. in height. The overlying skin was vascular but not adherent to the mass. Over certain places digital pressure elicited an egg-shell type of crackling. The mass was intimately attached to the upper part of the sternum.

The red blood cells numbered 4,500,000; the hemoglobin was 76 per cent (Tallqvist); the white blood cells numbered 9,200, with 68 per cent polymorphonuclears, 30 per cent lymphocytes and 2 per cent eosinophils. The Wassermann reactive of the blood was negative. Urinalysis gave negative results. Roentgen

examination of the chest revealed a tumor attached to and apparently arising from the manubrium sterni. It showed irregular foci of calcification. The clavicles were not involved.

A diagnosis of probable chondrosarcoma was made, and the manubrium was removed by Dr. Arthur M. Dickinson. The specimen consisted of a roughly spherical mass 10 by 9 by 7 cm. Irregular fragments of striated muscle and subcutaneous tissue were still adherent to the anterior surface of the manubrium. On the posterior surface there was a soft, pearly, opalescent tumor, friable and gelatinous, which was found to spring from a similar but more vascular neoplasm within the marrow space. Histologically, the tumor proved to be a myeloma of the plasma cell type. Careful clinical examination failed to reveal any of the usual symptoms of multiple myeloma. There was no anemia, evidence of renal impairment or Bence-Jones protein in the urine, and at no time had there been pain. The patient improved for four days, and then contracted acute bronchopneumonia of which he died. Permission for necropsy was refused, but roentgen examination of every part of the osseous system was made, and no evidence of involvement of the bone in any other part of the body was discovered.

Solitary myeloma is not common. To date only a few cases have been reported. Stewart and Taylor think that such cases may constitute a well defined clinical and pathologic group. Geschickter and Copeland, however, reported the case of a man who presented solitary myeloma of the clavicle. Subsequently the ribs and skull became involved, and the typical picture of multiple myeloma developed. The possibility that solitary myeloma is merely an early stage of multiple myeloma must not be forgotten.

UNSUSPECTED HEMOCHROMATOSIS, WITH MUCOCELE OF THE APPENDIX. GUSTAVUS H. KLINCK, JR.

A white woman, aged 46, for ten years had experienced gastric disturbances suggesting chronic pyloroduodenal ulcer. A gastro-enterostomy was performed by Dr. A. W. Elting. At the same time the appendix with a massive mucocele was removed. The appendix was 18 cm. in length and about 2.75 cm. in diameter. There was stenosis at the base of the lumen. Following operation the patient did well for a day, but died a few days later of what appeared to be an obscure vasomotor disturbance. There was no history of diabetes, nor was there hyperglycemia while the patient was in the hospital. At autopsy, the liver and pancreas presented a striking picture of hemosiderosis. The liver weighed 2,060 Gm., had sharp edges, was of firm consistence and was rusty brown. A fine irregularity of the surface suggested early cirrhosis. The organ cut with increased resistance. Close inspection showed a finely lobulated parenchyma with small islets of the hepatic tissue about 3 mm. in diameter standing slightly above the surrounding surfaces.

Microscopic examination revealed periportal cirrhosis of widespread distribution, with the formation of pseudolobules. There was no intracellular hyalin. Most striking was the intense siderosis, practically every hepatic cell containing some of the pigment. Sections stained with aqueous hematoxylin (Mallory) revealed fine copper granules in many of the islands of regenerating hepatic tissue. Copper granules were present in the secretion in the bile ducts, and they were found also in the smooth muscle of some of the hepatic arteries. The prussian blue reaction and the ammonium sulphide tests for iron were strongly positive on the bulk of the hepatic pigment.

The pancreas was of normal size, rather firm and chestnut brown. The parenchyma was finely granular, and the lobules were quite small. Numerous large grayish-brown lymph nodes were found posterior to the pancreas. Microscopic examination showed moderate interstitial fibrosis with marked hemosiderosis. The islets were normal, except that hemosiderin was present in small amounts in the epithelium. The corium of the skin of the abdomen contained a few granules of hemosiderin.

DISCUSSION

ELLIS KELLERT: Would incineration be of use in demonstrating the presence of copper?

V. C. JACOBSEN: Incinceration should be a valuable means of studying the distribution of iron in the liver in hemochromatosis, but it would probably be difficult to differentiate copper from iron by this method.

ARGYRIA WITH A GIGANTIC STONE IN THE BLADDER. GUSTAVUS H. KLINCK, JR.

A white woman, aged 55, deaf, entered the hospital because of pain in the bladder and incontinence of urine.

Physical examination revealed a marked bluish-gray discoloration of all the cutaneous surfaces, but most marked on the face and the forehead. The mucous membranes of the nose and mouth showed a similar ashen gray pigmentation. The sclerae were dull and blue-gray, and the conjunctivae were also pigmented. A roentgenogram showed a dense shadow occupying the area of the bladder. The patient was operated on by Dr. E. P. McDonald, and a stone in the bladder was removed, which weighed 275 Gm., was generally round and measured 10 cm. in diameter. It was rather rough and dark red, and when the surface was chipped off, a pale yellowish rocklike material was found to compose the stone. Portions of the stone were examined chemically for silver, but showed no trace of the metal. Frozen sections of a piece of skin from the abdomen showed black, slightly refractile granules abundant in and about the sweat glands in the typical manner of the granules of argyria. They remained uncolored with the prussian blue stain, but became blacker with ammonium sulphide. These granules appeared black in sections stained with hematoxylin and eosin, as well as in those stained with ammonium sulphide (heavy). The patient was carefully questioned with the view of determining the source of the argyria, but it could be learned only that she had taken pills of an unknown nature and a lemon-colored liquid about 7 years before admission over an undetermined period. On being examined by a physician about five years before admission, she was found to have an ashen gray color. Further history of the taking of drugs was not obtainable.

Book Reviews

Calcium Metabolism and Calcium Therapy. By Abraham Cantarow, M.D., Instructor in Medicine, Jefferson Medical College; in charge of Laboratory of Biochemistry, Jefferson Hospital; Assistant Physician, Philadelphia General Hospital. With a Foreword by Hobart Amory Hare, B.Sc., M.D., LL.D., Late Professor of Therapeutics, Materia Medica and Diagnosis, Jefferson Medical College, Philadelphia. Second edition. Price, $2.50, net. Pp. 252. Philadelphia: Lea & Febiger, 1933.

This monograph is a compilation and evaluation of recent contributions to the knowledge of the metabolism of calcium and its use in treatment. As Hare states in the foreword: "The present use of calcium is, in many instances, the introduction of an agent of which the physician knows little into a body of which he knows less, in entire forgetfulness of the universal rule that any agent capable of doing good is capable, if improperly used, of doing harm."

The subject matter is discussed under three heads: (1) normal calcium metabolism, (2) abnormal calcium metabolism and (3) calcium therapy. In the first section there is a discussion of the requirements, absorption and excretion of calcium and of various factors affecting the level of the serum calcium, as well as a review of the function of calcium, particularly as it affects the formation of bone, the permeability of the membranes and the excitability of nerves and muscles. Cantarow emphasizes the state of the calcium in the blood, and stresses the value of studying the diffusible and nondiffusible fractions of the serum calcium, which are in a sort of equilibrium and may vary independently of each other. He considers the estimation of calcium in the spinal fluid as a good method for determining the diffusible fraction of the serum calcium.

In view of the interest in vitamin D and parathormone, the chapter on the recent advances in this field merits consideration. There is a clear discussion of the inorganic metabolism in rickets and of the mode of action of vitamin D in its treatment. The toxic effects of overdosage with viosterol are described. However, the author notes that this should not interfere with the therapeutic use, since the occurrence of toxic manifestations with therapeutic doses of vitamin D is rare. Since surgeons have become interested in the use of calcium, parathyroid hormone and vitamin D in stimulating the healing of fractures, it is noteworthy that the author finds no physiologic or experimental basis for the use of these drugs to stimulate the healing of fractures. The clinical types of tetany are discussed as to the calcium metabolism, and the physiologic basis for treatment is explained. Cantarow notes that all forms of tetany are associated with hypocalcemia, which may be induced in different ways.

Modern calcium therapy is described. The dosage and pharmacologic properties of calcium salts and of parathyroid hormone, as well as the indications for their use, are stated clearly.

The monograph is a convenient compilation of the recent significant data on calcium metabolism, and should be of use to clinicians and investigators alike.

Sex and Internal Secretions: A Survey of Recent Research. By Edgar Allen, C. B. Bridges, C. H. Danforth, E. A. Doisy, L. V. Domm, E. T. Engle, R. G. Gustavson, C. G. Hartman, F. L. Hisaw, Mary Juhn, F. C. Koch, F. R. Lillie, C. R. Moore, J. P. Pratt, Oscar Riddle, A. E. Severinghaus, P. E. Smith, C. P. Stone, C. A. Turner, B. H. Willier, Emil Witschi. Edited By Edgar Allen, University of Missouri. With a Foreword by Robert M. Yerkes, Yale University. Price, $10. Pp. 951. Baltimore: Williams & Wilkins Company, 1933.

The history of this noteworthy book is briefly as follows: In 1921 the Bureau of Social Hygiene in New York made it possible to start a program of research on fundamental problems of sex under a committee of the Division of Medical Sciences of the National Research Council in Washington. To quote from the

foreword: "During the decade which has elapsed . . . scores of investigators have been assisted by financial aid, by suggestion, recommendation and other forms of advice. Publications in which committee assistance is recognized now approximate five hundred. Many notable discoveries and a vast contribution of fact, principally in the realms of the general biology, physiology, and psychobiology of sex, have been recorded." After ten years of continuous and successful effort, the committee, in taking stock within its field of interest, wisely decided to secure a comprehensive, cooperative survey of recent investigations on internal secretion in relation to sex. The result is the timely, well edited volume being reviewed. It is not possible to review the contents in detail chapter by chapter. In order to give a fair idea of the contents, it seems necessary, however, to enumerate at least the headings of the various chapters, each of which has an adequate bibliography: general biologic introduction; interrelation of genic and endocrine factors in sex; genetics of sex in Drosophila; embryologic foundations of sex in vertebrates; sex durations, inversions and parabiosis; metabolism and sex; biology of the testis; biochemistry and assay of testis hormone; ovarian follicular hormone, theelin and animal reactions; biochemistry of theelin; physiology of corpus luteum; the mammary glands; plumage tests in birds; ovulation and the transport of and viability of ova and sperm in the female genital tract; effect on the reproductive system of ablation and implantation of the anterior hypophysis; effects of extracts of the anterior pituitary and similar active principles of blood and urine; anterior pituitary changes referable to the reproductive hormones, and the influence of the thyroid and the suprarenals on genital function; sexual drive; endocrine disorders in sex functions in man. The book is the product of a well directed group effort by investigators of standing in their respective lines of work. It is an authoritative work of reference to recent researches on fundamental problems in its field, in which rapid progress is under way.

Neuropathology: The Anatomical Foundation of Nervous Diseases. By Walter Freeman, M.D., Ph.D., D.N.B., F.A.C.P., Professor of Neurology, George Washington University; Director of Laboratories, St. Elizabeth's Hospital, Washington, D. C. Cloth. Price, $4, net. Pp. 349, with 116 illustrations. Philadelphia: W. B. Saunders Company, 1933.

This book offers a summary of the knowledge of the morphologic changes in disease of the nervous system. It begins with an introductory chapter on the normal and pathologic morphology of the ganglion cell, the nerve fiber and its sheath, the neuroglia, the ependyma and the choroid plexus, and the connective tissue and lymphatic spaces. Included in this chapter are sections on the epiphysis and hypophysis, on products of degeneration and on postmortem changes and artefacts. Chapters 2 and 3 deal with the meninges and diseases of them and with vascular disease and arteriosclerosis. Then follow chapters on inflammations of the brain, cord and nerves; tuberculosis; syphilis; leprosy, fungous infections and parasites; intoxications; injuries; the functional psychoses, senile dementia and epilepsy; malformation; degenerations, and tumors. At the end is a glossary, and the usual subject index. Each chapter has a brief list of select references to appropriate recent literature. The 116 photographic illustrations are original and commendable. The descriptions of the morbid structural changes in the nerve tissues are clear, orderly, comprehensive and authoritative. Sometimes the writing appears a little hasty and unpolished. "Cornua ammona" is hardly acceptable for cornua ammonis. Is it necessary or desirable to fall back on foreign words, especially German, as much as we do to describe cellular forms in the nervous system? Why are so many burdensome, unneeded foreign words used? The inclusion of chorea and rheumatic fever in the rickettsial group of encephalitis does not seem warranted on etiologic grounds. As a whole, the book meets its purpose in full measure. It is an excellent review of the morphologic appearances in diseases of the nervous system. It is the product of a thorough familiarity with the literature of the subject and of an intensive and extensive study by modern methods of the structural lesions in nervous diseases. It will be a great help to students in this field everywhere.

Books Received

A Study of Nephritis and Allied Lesions. By John Gray, Medical Research Council, Special Report Series, No. 178. Price, 2 shillings, 6 pence, net. Pp. 141. London: His Majesty's Stationery Office, 1933.

La spirochétose méningée. Par Jean Troisier, Professeur agrégé à la Faculté de Médecine de Paris; Médecin de l'Hôpital Beaujon et Yves Boquien; Interne des Hôpitaux de Paris. Price, 34 francs. Pp. 188, with 22 figures. Paris: Masson et Cie, 1933.

Diseases of the Heart, Described for Practitioners and Students. By Sir Thomas Lewis, C.B.E., F.R.S., M.D., D.Sc., LL.D., F.R.C.P., Hon.D.Sc. (Michigan), Physician in Charge of Department of Clinical Research, University College Hospital, London; Physician of the Staff of the Medical Research Council; Physician-in-Chief (pro tem.), Peter Bent Brigham Hospital, Boston; Honorary Fellow, New York Academy of Medicine; Corresponding Member, Association of American Physicians and Interstate Postgraduate Medical Association. Price, $3.50. Pp. 297, with 45 figures. New York: The Macmillan Company, 1933.

Sex and Internal Secretions. Edited by Edgar Allen, University of Missouri. Price, $10. Pp. 951. Baltimore: The Williams & Wilkins Company, 1933.

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